(54) Title: METHODS AND COMPOSITIONS FOR MODULATING APOPTOSIS

(57) Abstract: This invention relates to compositions and methods for modulating apoptosis by regulating the activity of endoplasm
reticulum transmembrane glucose regulated protein 78 (GRP78).

Methods and Compositions for Modulating Apoptosis

CROSS REFERENCE TO RELATED APPLICATIONS

[0001]   This application claims priority under 35 U.S.C. §119 to U.S. Provisional Application Serial No. 60/514,661, filed October 27, 2003, the disclosure of which is incorporated herein by reference.

STATEMENT AS TO FEDERALLY-SPONSORED RESEARCH

[0002]   Pursuant to 35 U.S.C. §202(c), it is acknowledged that the U.S. Government has certain rights in the invention described herein, which was made in part with funds from the National Institutes of Health, Grant Nos. CA 20607 and AI42394.

TECHNICAL FIELD

[0003]   This invention relates to methods and compositions for modulating apoptosis by selectively targeting glucose regulated proteins (GRPs) and more particularly to modulating the activity and/or interaction of GRP78 and procaspase.

BACKGROUND

[0004]   Resistance to chemotherapy remains a major obstacle for the treatment of cancer. The complexity of drug resistance in human cancer strongly suggests the involvement of multiple pathways. One mechanism, both intrinsic and acquired, is the result of genetic alterations within cancer cells. Another mechanism may result from environmental conditions that occur naturally in solid tumors. Because of poor vascularization, solid tumors usually contain regions undergoing glucose starvation and hypoxia, resulting in acidosis and alterations in cell metabolism. These pockets of hypoxia and nutrient deprivation occur in well differentiated, slow growing, non-metastatic tumors, as well as in rapidly growing, aggressive anaplastic malignancies.

[0005]   Stress conditions in cell culture, such as glucose starvation, commonly cause the glucose-regulated stress response which, is part of a general cellular defense mechanism referred to as the unfolded protein response (UPR). One characteristic of the UPR is the induction of the endoplasmic reticulum (ER) resident stress proteins referred to as the glucose-regulated

proteins (GRPs). The GRPs are Ca^{2+}-binding chaperone proteins
with protective properties. The best characterized GRP is GRP78,
a 78-kDa protein also referred to as BiP. As a protein
chaperone, GRP78 is known to form complexes with heterologous
proteins that are processed through the ER.

[0006]   Glucose Regulated Proteins, or GRPs (GRP74, GRP78,
GRP94, GRP170, ERp72, PDI, calreticulin, and GRP58 (alias ERp57))
are ER molecular chaperones that assist in protein folding and
assembly. GRP78, GRP94, ERp72 and calreticulin are also Ca2+
binding proteins. GRP78 and GRP94 share sequence homology with
heat shock proteins. The GRP family of proteins is coordinately
induced by glucose starvation, anoxia, alterations in
intracellular calcium and, exposure to inhibitors of
glycosylation as well as by PDT-mediated oxidative stress (Gomer,
et al., Cancer Res. 51:6574-79, 1991; and Li, et al., J. Cell
Physiol. 153:575-82, 1992). The 78,000 GRP (i.e., GRP78) is
identical in sequence to the immunoglobulin heavy chain binding
protein and both GRP78 and GRP94 are localized in the ER.

[0007]   Many of the cytotoxic drugs, including topoisomerase
inhibitors such as etoposide, initiate programmed cell death.
DNA damaging agents such as etoposide can trigger cell death
through the p53-mediated caspase cell death signaling cascade,
resulting in cytochrome c release and the activation of caspase-
3. Caspases-3, -6, and -7 are members of the apoptotic executing
group of caspases with caspase-7 structurally and functionally
most similar to caspase-3. Active caspase-7 has been shown to be
associated with the mitochondria and the ER membranes, whereas
caspase-3 remains cytosolic. Although these observations suggest
that similar apoptotic executioner's function in different
cellular compartments and act on distinct substrates, there is
limited information on the contribution of organelles such as the
ER in the apoptotic process.

[0008]   Accordingly, there exists a need in the art to
identify key interactions between proteins involved in the
apoptotic pathway and to regulate those interactions.

SUMMARY

[0009]   Overexpression and antisense approaches in cell
systems show that GRP78 can protect cells against cell death
caused by disturbance of ER homeostasis.   Whereas GRP78
overexpression could limit damage in normal tissues and organs
exposed to ER stress, the anti-apoptotic function of GRP78 also
predicts that its natural induction in neoplastic cells could
lead to cancer progression and drug resistance.   In a variety of
cancer cell lines, solid tumors, and human cancer biopsies, the
level of GRP78 is elevated, correlating with malignancy. Using
human cancer and other cell lines, a large number of stress
induction studies show that a glucose-regulated stress response
results in the induction of GRP78 and other coordinately
regulated GRP genes correlating with cellular drug resistance.
Nonetheless, the direct role of GRPs in conferring drug
resistance has not been proven. This is because of the inherent
problems associated with using stress inducers or deficiencies in
certain cell functions to induce the GRPs, because the inducing
conditions can exert other unknown pleiotropic effects, possibly
affecting multiple cellular pathways.   Furthermore, the
mechanisms for the protective function of the ER localized GRPs
in drug resistance are not understood.

[0010]   Methods and compositions for modulating apoptosis by
regulating the physical and functional interactions of glucose
responsive protein 78 (GRP78) with cytosolic components of a cell
that mediate apoptosis are provided. The methods and
compositions are particularly well suited to identifying agents
that can be used in conjunction with apoptosis-inducing
therapeutic compounds to treat cell proliferative disorders.
Thus, the disclosure relates to the preparation of pharmaceutical
compositions for treating, preventing, and/or delaying a disease
in a subject, such as, for example, a cell proliferative
disorder.

[0011]   Accordingly, in one embodiment, the invention provides
a method of modulating apoptosis by contacting glucose regulated
protein 78 (GRP78) endoplasmic reticulum transmembrane protein
with an agent that regulates the interaction of the transmembrane
protein with a cytosolic component that mediates apoptosis. In

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one aspect, the cytosolic component is a caspase. The caspase
can be, for example, caspase-7. In general, the method can be
used to promote or inhibit apoptosis. The agent can be, for
example, a polypeptide, an antibody or a small molecule. In one
aspect, the agent interacts with the ATP-binding domain of GRP78.

[0012]   In another embodiment, the invention provides a method
of modulating apoptosis by contacting glucose regulated protein
78 (GRP78) with an agent that inhibits or prevents the ability of
the protein to integrate in to the membrane of the endoplasmic
reticulum. In one aspect, the agent interacts with a hydrophobic
transmembrane domain III (amino acids 210-260 of SEQ ID NO:2) or
domain IV (amino acids 400-450 of SEQ ID NO:2) of GRP78.

[0013]   In a further embodiment, the invention provides a
method of identifying an agent that modulates the interaction of
glucose regulated protein 78 (GRP78) with a cytosolic component
that mediates apoptosis. The method includes providing glucose
regulated protein 78 (GRP78) integrally-associated with a
membrane, providing a cytosolic component comprising at least one
caspase, providing an agent, contacting the protein with the
component and the agent simultaneously or in succession, and
determining the effect of the agent on the interaction of the
protein and the component as compared to a control.

[0014]   In a further embodiment, the invention provides a
method of identifying an agent that modulates the interaction of
glucose regulated protein 78 (GRP78) with a membrane. The method
includes providing a polypeptide comprising the hydrophobic
transmembrane domain III (amino acids 210-260 of SEQ ID NO:2)
and/or domain IV (amino acids 400-450 of SEQ ID NO:2) of the
protein of glucose regulated protein 78 (GRP78), providing an
agent, contacting the polypeptide with the agent, and determining
the effect of the agent on the ability of the polypeptide to
incorporate in to the membrane, as compared to a control.

[0015]   In yet another embodiment, the invention provides a
method of inhibiting apoptosis in a target tissue by
overexpressing GRP78 or GRP94 in said tissue. Exemplary tissues
include neuronal tissue, vascular tissue and cardiac tissue.

[0016]   The invention provides a method of modulating
apoptosis in a cell, the method comprising contacting a glucose

regulated protein (GRP) with an agent that regulates the interaction of the GRP with a cytosolic component that mediates apoptosis.

[0017] The invention also provides a method of promoting apoptosis in a cell, the method comprising inhibiting glucose regulated protein 78 (GRP78) with an agent that (i) inhibits or prevents the ability of GRP78 to interact with a cytosolic protein and/or (ii) inhibits the production of GRP78.

[0018] The invention further provides a method of identifying an agent that modulates the interaction of glucose regulated protein 78 (GRP78) with a cytosolic component that mediates apoptosis. The method comprises (a) providing glucose regulated protein 78 (GRP78) integrally-associated with a membrane; (b) providing a cytosolic component comprising at least one caspase; (c) providing an agent; (d) contacting the protein of a) with the component of (b) and the agent of (c) simultaneously or in succession; and (e) determining the effect of the agent on the interaction of the protein and the component as compared to a control.

[0019] The invention provides a glucose regulated protein (GRP) inhibitory nucleic acid molecule comprising a nucleic acid that interacts with a glucose regulated protein (GRP) polynucleotide. In one aspect, the inhibitory nucleic acid is an antisense molecule. In another aspect, the nucleic acid is a small inhibitory nucleic acid (siNA) molecule.

[0020] The invention also provides a glucose regulated protein modulating agent comprising a soluble domain of a GRP protein.

[0021] The invention further provides a method of identifying an agent that modulates the interaction of glucose regulated protein 78 (GRP78) with a cytosolic component that mediates apoptosis, the method comprising: (a) providing a polypeptide comprising the ATP-binding domain of glucose regulated protein 78 (GRP78); (b) providing a cytosolic component comprising at least one caspase; (c) providing an agent; (d) contacting the polypeptide of a) with the component of (e) and the agent of (c) simultaneously or in succession; and (f) determining the effect

of the agent on the interaction of the polypeptide and the component as compared to a control.

[0022]   The invention yet further provides a method of identifying an agent that modulates the interaction of glucose regulated protein 78 (GRP78) with a membrane, the method comprising: (a) providing a polypeptide comprising the hydrophobic transmembrane domain III (amino acids 210-260 of SEQ ID NO:1 or 2) and/or domain IV (amino acids 400-450 of SEQ ID NO:1 or 2) of the protein of glucose regulated protein 78 (GRP78); (b) providing an agent; (c) contacting the polypeptide of a) the agent of b) simultaneously or in succession; and (d) determining the effect of the agent on the interaction of the polypeptide with the membrane as compared to a control.

[0023]   The invention provides a method of modulating apoptosis, the method comprising contacting a cell comprising a caspase polypeptide with an agent that regulates the interaction of the polypeptide with glucose regulated protein 78 (GRP78) endoplasmic reticulum transmembrane protein.

[0024]   The invention also provides a method of modulating apoptosis, the method comprising contacting a cell comprising a glucose regulated protein 94 (GRP94) endoplasmic reticulum transmembrane protein with an agent that regulates the interaction of the transmembrane protein with a cytosolic component that mediates apoptosis.

[0025]   The invention further provides a method of inhibiting apoptosis in a target tissue, the method comprising overexpressing GRP78 or GRP94 in said tissue.

[0026]   The invention provides a method of identifying an agent that modulates the interaction of glucose regulated protein 94 (GRP94) with a cytosolic component that mediates apoptosis, the method comprising: (a) providing glucose regulated protein 94 (GRP94); (b) providing a cytosolic component comprising at least one caspase; (c) providing an agent; (d) contacting the protein of (a) with the component of (b) and the agent of (c) simultaneously or in succession; and (e) determining the effect of the agent on the interaction of the protein and the component as compared to a control.

[0027]   Also provided by the invention is a nucleic acid construct comprising a glucose regulated protein (GRP) inhibitory nucleic acid molecule operably linked to an expression control element.

[0028]   In another aspect, the invention provides a recombinant vector comprising a nucleic acid construct comprising a glucose regulated protein (GRP) inhibitory nucleic acid molecule operably linked to an expression control element.

[0029]   The invention provides a pharmaceutical composition comprising a nucleic acid construct of the invention in a pharmaceutically acceptable carrier.

[0030]   A method for inhibiting cell proliferation is also provided by the invention. The method comprising contacting a target cell having a cell proliferative disorder with a nucleic acid construct of the invention.

[0031]   In yet another aspect the invention provides a method for treating a cell proliferative disorder in a subject comprising administering to the subject a nucleic acid construct of the invention.

[0032]   The invention provides a nucleic acid construct comprising a glucose regulated protein (GRP) polynucleotide operably linked to an expression control element.

[0033]   The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

[0034]   **Figure 1, Panel A** depicts a comparison of GRP78 protein levels in parental CHO cell line and its derivative C.1. Total protein lysates (25 and 50 μg/lane) were separated on 8% SDS-PAGE, and the levels of GRP78, GRP94, and protein X were determined by immunoblotting with an anti-KDEL antibody.

[0035]   **Figure 1, Panel B** depicts GRP78 localization by immunofluorescence. Subcellular distribution of GRP78 is primarily perinuclear, indicative of ER localization.

[0036]   **Figure 1, Panel C** depicts co-localization of GRP78 and caspase-7 in the ER. Green fluorescence indicates caspase-7, red

indicates GRP78, and purple staining represents the nucleus.   In
the merged images, yellow staining indicates co-localization.

[0037]   **Figure 2, Panel A** depicts clonogenic survival assays
for CHO and C.1 cells subjected to various concentrations of
etoposide for 6 h.

[0038]   **Figure 2, Panel B** depicts clonogenic survival assays
for CHO and C.1 cells subjected to various concentrations of
adriamycin for 1 h.

[0039]   **Figure 2, Panel C** depicts clonogenic survival assays
for CHO and C.1 cells subjected to various concentrations of
camptothecin for 24 h.

[0040]   **Figure 3, Panel A** depicts GRP78 overexpression
protecting cells from etoposide-induced apoptosis.   C.1 and CHO
cells were either non-treated (Ctrl) or treated with 30 μM
etoposide (Etop) for 6 h.   Viable cells are those with low
annexin or no annexin and PI staining (lower left panel). Early
stage apoptotic cells are represented by high annexin and low PI
staining (lower right panel), later stage apoptotic cells
represented by high annexin and high PI staining (upper right
panel), and necrosis is represented by cells with high PI and low
annexin staining (upper left panel).

[0041]   **Figure 3, Panel B** depicts DNA fragmentation pattern of
CHO and C.1 cells following etoposide treatment.

[0042]   **Figure 4, Panel A** depicts overexpression of GRP78
confers etoposide resistance to human bladder carcinoma T24/83
cells.   T24/83 cell lines stably transfected with the empty
vector pcDNA (T24/83-pcDNA) or vector expressing wild-type GRP78
(T24/83-GRP78) were established.   Immunoblot analysis of GRP78
protein level for the two cell lines is shown (inset).

[0043]   **Figure 4, Panel B** depicts immunofluorescence imaging
of GRP78 expression in T24/83 cells.   GRP78 localization is
perinuclear.

[0044]   **Figure 4, Panel C** depicts the effect of overexpression
of GRP78 on etoposide-induced apoptosis.   T24/83-pcDNA and
T24/83-GRP78 cells were either non-treated (Ctrl) or treated with
etoposide (Etop).

[0045]   **Figure 5, Panel A** depicts the effect of GRP78
overexpression on topoisomerase II expression and caspase-7

activation. Total protein lysates were prepared from non-treated
(Ctrl) or etoposide-treated (Etop) CHO and C.1 cells.

[0046]   Figure 5, Panel B depicts overexpression of GRP78 as
inhibiting in vitro activation of caspase-7.

[0047]   Figure 5, Panel C depicts cytoplasmic extracts
prepared from CHO and C.1 cells and incubated with (+) or without
(-) 10 µM cytochrome c and the various amounts of dATP (mM) as
indicated.

[0048]   Figure 6, Panel A depicts cell lysates from CHO and
C.1 cells immunoprecipitated with anti-caspase-7 (lanes 1 and 2)
or anti-caspase-3 (lanes 3 and 4) antibodies.

[0049]   Figure 6, Panel B depicts cell lysates in extraction
buffer from CHO and AD-1 cells were immunoprecipitated with anti-
caspase-7 antibody (lanes 1 and 2). Whole cell extracts (WCE)
from CHO and AD-1 cells were immunoblotted in parallel (lanes 3
and 4). The positions of GRP78, procaspase-7, and the deletion
mutant form of GRP78 (Δ78) are indicated.

[0050]   Figure 6, Panel C depicts a schematic drawing of wild-
type (WT) GRP78 and AD-1 showing the location of the signal
sequence (S), the ATP-binding domain, and the AD-1 deletion
spanning amino acids 175 to 201.

[0051]   Figure 7, Panel A depicts a hydropathicity plot of
GRP78 as generated using the Kyte-Doolittle method with a window
size of 17. Four putative hydrophobic domains (I-IV) are
identified. Represented below is a schematic drawing of the
mature GRP78 protein with the hydrophobic domains IV and III as
putative transmembrane domains generating carboxyl 35- and 50-kDa
trypsin resistant fragments.

[0052]   Figure 7, Panel B depicts limited trypsin digestion.
Isolated microsomes from C.1 cells were either non-treated (lane
1) or subjected to trypsin digestion at the concentration of
0.01% (lane 2) or 0.05% (lane 2). At the end of the reaction,
the amount of GRP78 was detected by Western blotting using the
rabbit polyclonal anti-GRP78 antibody recognizing the carboxyl
terminus (StressGen, Victoria, Canada) (left panel). The full-
length GRP78 band is indicated by a closed arrow, and the 35- and
50-kDa proteolytic products are indicated by open arrows
highlighted with a star. The same membrane was stripped and re-

probed with a rabbit polyclonal anti-calreticulin (CRT) antibody
(middle panel) or a rabbit polyclonal anti-calnexin antibody
recognizing the amino terminus of calnexin (right panel). The
full-length CRT and calnexin are indicated by closed arrows, and
the 70-kDa proteolytic product of calnexin is indicated by an
open arrow highlighted by a star.

[0053]   Figure 7, Panel C depicts sodium carbonate extraction.
The microsome (M) fraction was either non-treated (lane 1) or
treated with 100 mM sodium carbonate and separated into pellet
(P) and supernatant (S) fractions (lanes 2 and 3, respectively).
The protein samples from each fraction were separated by 10% SDS-
PAGE and subjected to Western blotting with rabbit anti-GRP78
antibody (left panel), rabbit anti-calreticulin antibody (middle
panel), and rabbit anti-calnexin antibody (right panel).

[0054]   Figure 8, Panel A depicts cytoplasmic extracts (50
µg/lane) prepared from non-treated (Ctrl) and etoposide-treated
(Etop) AD-1 and C.1 cells as separated on 10% SDS-PAGE and
immunoblotted using anticaspase-7 antibody.

[0055]   Figure 8, Panel B depicts the DNA fragmentation
pattern of C.1 and AD-1 cells following etoposide treatment.

[0056]   Figure 8, Panel C depicts cell death assays indicating
that the ATP-binding domain of GRP78 is necessary for protection
against etoposide-induced cell death. The S.D. is shown.

[0057]   Figure 9 depicts the relative cell cycle distribution
of GRP-78. The percentage of cells in G1, G2, and S phase was
determined.

[0058]   Figure 10 is a schematic showing the subcloning of the
320 bp grp78 exon 1 fragment.

[0059]   Figure 11A-C shows the schemes used for subcloning.
(A) shows the scheme for the subcloning of full-length His-tagged
GRP78 into pShuttle CMV adenovirus; (B) shows the scheme for the
subcloning of full-length His-Tagged GRP78 Full-length antisense
(AS); and (C) shows the scheme used for the subcloning of GRP78
(320BP) antisense (AS) into PShuttle CMV adenovirus.

[0060]   Figure 12 shows the expression of His-tagged GRP78,
GRP78(AS) and 320(AS) in adenovirus infected 293 T cells.

[0061]   Figure 13A and B shows the results of transduced MDA-
MB-435 cells treated with etoposide in the presence and absence
of the 320(AS).

[0062]   Figure 14 shows a Western blot of 293T cells. The
concentrations of siRNA Grp78 II used were indicated on top.

[0063]   Figure 15 shows a Western blot of MDA-MB-435 cells.
The concentrations of siRNA Grp78 II used were indicated on top.

[0064]   Figure 16 shows suppression of GRP78 by siRNA
oligonucleotides results in enhanced sensitivity to etoposide-
mediated cell death in breast cancer cells.

[0065]   Figure 17A-B show the physical and functional
interaction between GRP BlK. (A) 293T cells were either not
treated (lanes 1, 2) or treated with 50 uM etoposide for 24 hours
(lane 3). Cells were harvested and cells lysate
immunoprecipitated with either goat IgG (lane 1) or goat anti-BlK
antibody (lanes 2, 3). Western blots with anti-BlK antibody and
anti-GRP78 antibody show co-immunoprecipitation of endogenous
GRP78 with Blk. (B) 293T cells were transiently transfected with
either pcDNA, CMV promoter driven expression vector for His-
tagged GRP78, expression vector for Flag-tagged Glk-b5TM alone or
in combination as indicated. After 48 hours, cell death was
determined by trypan blue exclusion assay. The level of
apoptosis observed in cells transfected with pcDNA3 was set as 1.
The results showed overexpression of GRP78 protects 293T cells
from death induced by transient transfection of ER-targeted Blk-
b5tM.

DETAILED DESCRIPTION

[0066]   Within the microenvironment of a solid tumor, unique
stress conditions can lead to induction of GRPs. The data and
invention described herein indicate that GRPs act as anti-
apoptotic proteins. The GRP-mediated protection involves GRP
interaction with effectors of apoptosis, leading to the blockage
of cell death induced by drug treatment.

[0067]   Thus, in accordance with the invention, GRPs (e.g.,
GRP74, GRP78 and GRP94) represent rational targets for
chemotherapeutics, immunotherapeutics, antisense, ribozymes,
siRNA and vaccines relevant to the treatment of cell
proliferative diseases such as cancer. In view of their function

as molecular chaperones, the GRPs (e.g., GRP78 and 94) further
represent rational targets for the development of therapeutics
for tissue injury and stress, such as can occur in ischemic
injuries including, but not limited to, organ (kidney, heart,
lung, liver) transplantation, cerebral stroke, and myocardial
infarct. Methods and compositions for modulating apoptosis are
provided.

[0068]   The invention provides methods and compositions useful
to modulate apoptosis in a cell, tissue and/or subject.
"Apoptosis" refers to programmed cell death which occurs by an
active, physiological process. Apoptosis plays an important role
in developmental processes, including morphogenesis, maturation
of the immune system, and tissue homeostasis whereby cell numbers
are limited in tissues that are continually renewed by cell
division. Apoptosis is an important cellular safeguard against
tumorigenesis. An apoptotic cell or a cell going through
"programmed cell death" exhibits one or more characteristics
associated with timed or targeted cell death. Characteristics
include inhibition of cell survival, growth, death or
differentiation, protein/nucleic acid cleavage/fragmentation,
chromatin condensation, membrane fragmentation, changes in
expression or activity of one or more proteins that promote
apoptosis or that inhibit apoptosis.

[0069]   "Modulating" apoptosis means increasing, stimulating
or inducing, or decreasing, inhibiting, blocking or preventing
(e.g., prophylaxis) one or more characteristics of programmed
cell death as described herein or known in the art. For example,
the methods and compositions of the disclosure include agents
(e.g., antisense molecules, ribozymes, polypeptides, small
molecules, and the like) that increase, stimulate or induce
apoptosis by inhibiting the activity or production of GRPs. The
disclosure also includes agents and methods that increase the
activity or production of GRPs to inhibit apoptosis in tissues or
cells subject to damage due to ischemia and the like.

[0070]   GRP78 binds transiently to nascent, secretory and
transmembrane proteins and binds permanently to abnormally folded
or processed proteins in the ER. GRP78 is thought to have a
protective function during and after cellular stress when protein

processing in the ER is perturbed. GRP78 has been proposed as a possible target for several antitumor agents, principally radicicol and geldanamycin (Scheibel and Buckner, Biochem Pharm 56:675-82, 1998).

[0071]   A potential yet heretofore uncharacterized protective role of GRP94 in ischemia is supported by the observation that expression of GRP94 is enhanced in hippocampus after transient forebrain ischemia of a duration known to result in neuronal death (Yagita et al., J Neurochem 72:1544-1551, 1999). GRP94 is similarly induced following acute kidney ischemia (Kuznetsov, Proc Natl Acad Sci USA 93:8584-8589, 1996). By comparison, heat-shock proteins, including HSP90, are over-expressed during the oxidative stress of reperfusion that generally follows ischemia. For example, the higher levels of GRP78 and GRP94 in the brains of immature rats when compared to those of adult animals account for the higher resistance of immature rats to seizure. In addition, specific induction of these GRPs in the dentate gyrus region of the adult rat brain following seizure is associated with a neuroprotective effect. For early-onset familial Alzheimer's disease (FAD), overexpression of GRP78 in neuroblastoma cells expressing a mutant presenilin-1 (PS1) protein was reported to restore resistance to ER stress.

[0072]   Expression of GRP78 also prevents the aggregation and facilitates the proteasomal degradation of mutant prion proteins, which are implicated in neurodegenerative disorders such as prion diseases and transmissible spongiform encephalopathies. Induction of GRP78 has also been observed in endothelial cells damaged by reductive stress that was caused by hyperhomocysteinaemia, which, with both genetic and environmental components, is a common risk factor for thrombotic vascular events such as premature arteriosclerosis, stroke, myocardial infarction, and thrombosis. Therefore, the induction of GRP could be an adaptive response evolved in mammals to protect endothelial cells against stress-induced cell death.

[0073]   Although apoptosis is mediated by diverse signals and complex interactions of cellular gene products, the results of these interactions ultimately feed into a cell death pathway that is evolutionarily conserved between humans and invertebrates.

The pathway, itself, is a cascade of proteolytic events analogous to that of the blood coagulation cascade.

[0074]   Several gene families and products that are involved in the apoptotic pathway have been identified. Key to the apoptotic program is a family of cysteine proteases termed caspases. The human caspase family includes Ced-3, human ICE (interleukin-1beta converting enzyme) (caspase-1), ICH-1 (caspase-2), CPP32 (caspase-3), ICErelII (caspase-4), ICErelII (caspase-5), Mch2 (caspase-6), ICE-LAP3 (caspase-7), Mch5 (caspase-8), ICE-LAP6 (caspase-9), Mch4 (caspase-10), caspase 11-14 and others.

[0075]   It has been demonstrated that caspases are required for apoptosis to occur. Moreover, caspases appear to be necessary for the accurate and limited proteolytic events that are the hallmark of classic apoptosis (see Salvesen and Dixit, Cell 91:443-446, 1997). During apoptosis, an initiator caspase zymogen is activated by autocatalytic cleavage, which then activates the effector caspases by cleaving their inactive zymogen (Salvesen and Dixit, Proc. Natl. Acad. Sci. USA 96:10964-10967, 1999; Srinivasula et al., Mol. Cell. 1:949-957, 1998). The effectors are responsible for proteolytic cleavage of a number of cellular proteins leading to the characteristic morphological changes and DNA fragmentation that are often associated with apoptosis (reviewed in Cohen, Biochem. J. 326:1-16, 1997; Henkart, Immunity 4:195-201, 1996; Martin and Green, Cell 82:349-352, 1995; Nicholson and Thomberry, TIBS 257:299-306, 1997; Porter et al., BioEssays 19:501-507, 1997; Salvesen and Dixit, Cell 91:443-446, 1997).

[0076]   Among the executor caspases, caspase-7 has been reported to be associated with the ER. Upon induction of apoptosis, procaspase-7 (35 kDa) is first converted into a 32-kDa intermediate, which is further processed into active 20- and 11-kDa subunits. Western blotting data provided herein indicates that treating CHO cells with etoposide results in activation of caspase-7, giving rise to the 32-kDa intermediate form (Figure 5, Panel A). Upon longer exposure of the autoradiogram, the active 20- and 11-kDa forms were evident in the etoposide-treated cells. When GRP78 was overexpressed, a low level of caspase-7 activation

was detected in both the non-treated and etoposide-treated cells. These data indicate that GRP78 can suppress caspase-7 activation *in vivo* thereby inhibiting apoptosis. In addition, upon addition of cytochrome c, caspase-7 activation was higher in CHO cells than C.1 cells, as evidenced by the increase in the active 20- and 11-kDa forms in the CHO samples compared with the C.1 samples. In the presence of both cytochrome c and dATP, the suppressive effect of the C.1 samples was reversed. At 1 mM dATP, both cell lines showed equivalent amounts of the 32- and 20-kDa forms, indicating that dATP releases procaspase-7 from GRP78, resulting in its activation.

[0077] The invention demonstrates that over-expression in tissue culture systems of GRP78, GRP94 and adapt78 can protect cells against cell death. The invention also demonstrates that inhibitors of expression (e.g., antisense and RNAi) or inhibitors of GRP activity in tissue culture systems can induce apoptosis. Thus, the protective function of the GRPs is useful and beneficial in situations involving tissue or organ damage. This same protective function is detrimental in cancer by preventing apoptosis of cancer cells.

[0078] As demonstrated herein, GRP upregulation and/or overexpression is useful in limiting damage in organs exposed to stress. However, the anti-apoptotic function of GRPs also indicates that their induction in neoplastic cells and cell proliferative disorders could lead to cancer progression and drug resistance. In a variety of cancer cell lines, solid tumors and human cancer biopsies, the levels of GRP78 and GRP94 are elevated, correlating with malignancy. In addition, induction of GRP78 has been shown to protect cancer cells from immune surveillance, whereas suppressing the stress-mediated induction of GRP78 enhanced apoptosis, inhibited tumor growth and increased the cytotoxicity of chronic hypoxic cells.

[0079] Thus, the invention provides methods and compositions useful for targeted suppression of GRP expression or function in cancer cells as a novel approach to cancer therapy. For example, Genistein, which suppresses both the GRP and the heat shock responses, inhibits the growth of carcinogen-induced tumors in rats and in human leukemia cells transplanted into mice. In

another example, GRP94 has been shown to associate with and
stabilize p185/erbB2 (also referred to HER-2/neu), which is
commonly over-expressed in breast carcinomas and is associated
with poor prognosis. Treatment of breast cancer cells with
geldanamycin, an anti-proliferative agent, enables the
degradation of p185 in the breast cancer cells by disrupting the
GRP94-p185 complex.

[0080]   Pre-induction of GRP in a variety of human cancer cell
lines confers resistance to inhibitors of topoisomerase II (e.g.,
etoposide) but increases sensitivity to DNA cross-linking agents
such as cisplatin. Direct suppression of GRP94 levels by
antisense knockdown strategies results in enhanced sensitivity to
etoposide-induced cell death.

[0081]   Accordingly, the invention provides methods and
compositions useful in reducing the anti-apoptotic effect of
GRPs, increase sensitivity of cancer cells to chemotherapeutic
agents, and promote apoptosis of neoplastic cells. The methods
and compositions of the invention inhibit the production or
activity of GRPs in neoplastic cells (e.g., cancer cells) and
tissues.

[0082]   As will be discussed below, the invention provides the
first evidence that a population of GRPs is integrally-associated
with the membrane of the endoplasmic reticulum. These GRPs
interact with a cytosolic component to mediate apoptosis. A
"cytosolic component that mediates apoptosis", as used herein, is
any polypeptide, or group of polypeptides that cooperate in the
initiation or facilitation of apoptosis. For example, the
interaction between GRP78 and caspase-7 and/or the interaction
between GRP94 and p185/erbB2 is involved in GRPs ability to
modulate apoptosis. The interaction can be, for example, with
caspase-7 individually, or as part of a group of other
polypeptides involved in the apoptosis pathway.

[0083]   The invention further provides the first evidence that
complex formation between endogenous GRP78 and caspase-7 occurs
in association with the endoplasmic reticulum. While the data
provided herein indicates that GRP78 and caspase-7 interact, the
invention is not limited to a direct interaction between the two
proteins. It is understood that the invention encompasses a

cytosolic component that is a complex of polypeptides, including caspase-7, or caspase-7 individually. By preventing the interaction of GRP78 with caspase-7, the agent modulates apoptosis by promoting apoptosis. Alternatively, by promoting the interaction of GRP78 with caspase-7, an agent would modulate apoptosis by inhibiting apoptosis.

[0084] In one embodiment, the invention provides a method of modulating apoptosis by contacting a GRP (e.g., GRP74, 78, and/or 94) with an agent that regulates the interaction of the GRP protein with a cytosolic component that mediates apoptosis. As used herein, the term "interact" includes any detectable interactions between molecules. The term "interact" is also meant to include "binding" interactions between molecules. Interactions can, for example, be protein-protein, protein-nucleic acid, and nucleic acid-nucleic acid in nature including hydrogen-bond interactions, covalent-bond interactions and the like.

[0085] An "agent", as used herein, can be any molecule including, for example, a polypeptide, an antibody, a nucleic acid (e.g., an antisense, ribozyme, siRNA or the like) or a small molecule. An agent can be a "therapeutic agent" useful for treating disorders associated with cell proliferation including anti-neoplastic agents and anti-inflammatory agents.

[0086] The invention provides apoptotic agents (e.g., GRP antagonists) comprising agents that inhibit the anti-apoptotic affect of GRPs (e.g., GRP78). In one aspect of the invention, a small molecule such as dATP is used to prevent the interaction, and/or disrupt the interaction of GRP78 with caspase-7 thereby inhibiting the anti-apoptotic activity of GRP78. In another aspect, agents are provided that inhibit transcription from a GRP (e.g., GRP78) gene. For example, versielostatin (VST) is useful to inhibit transcription from GRP78 (Park et al., J. Nat. Canc. Inst., 96(17):1300-1310, 2004; the disclosure of which is incorporated herein by reference). In another aspect, inhibitory nucleic acid molecules (e.g., antisense, ribozymes, siRNA) molecules are used to inhibit the production of GRPs (e.g., GRP78). In one aspect, the apoptotic agents provide a method for inducing apoptosis by inhibiting the production of GRP78 thereby

inhibiting the anti-apoptotic affect of GRP78 in a cell. The
apoptotic agents of the invention are useful in treating
neoplastic and cancer disorders by promoting apoptosis in cells
expressing GRPs such as GRP78.

[0087]   In embodiments where apoptosis is desired, the agent
that directly reduces expression/activity of GRP can be a nucleic
acid that reduces expression of GRP. In embodiments where anti-
apoptotic activity is desired, the nucleic acid can be a sense
nucleic acid that encodes a GRP protein (e.g., introduction into
a cell can increase the cells GRP activity).

[0088]   In one embodiment where apoptotic activity is desired
an apoptotic agent such as a GRP antagonist is used. In one
aspect, an apoptotic nucleic acid agent is used. An apoptotic
nucleic acid agent can be an antisense nucleic acid that
hybridizes to mRNA encoding a GRP. Antisense nucleic acid
molecules for use with the invention are those that specifically
hybridize under cellular conditions to cellular mRNA and/or
genomic DNA encoding a GRP protein in a manner that inhibits
expression of the GRP protein, e.g., by inhibiting transcription
and/or translation. The binding may be by conventional base pair
complementarity, or, for example, in the case of binding to DNA
duplexes, through specific interactions in the major groove of
the double helix.

[0089]   Antisense constructs can be delivered as an expression
plasmid which, when transcribed in the cell, produces RNA which
is complementary to at least a unique portion of the mRNA and/or
endogenous gene which encodes a GRP protein. Alternatively, the
antisense construct can take the form of an oligonucleotide probe
generated ex vivo which, when introduced into a GRP protein
expressing cell, causes inhibition of GRP protein expression by
hybridizing with an mRNA and/or genomic DNA coding for a GRP
protein. Such antisense molecules may comprise modified
nucleotides that are resistant to endogenous nucleases, e.g.,
exonucleases and/or endonucleases, and are therefore stable in
vivo. Additionally, general approaches to constructing oligomers
useful in antisense therapy have been reviewed, for example, by
Van der Krol et al., Biotechniques 6:958-976, 1988; and Stein et
al., Cancer Res. 48:2659-2668, 1988.

[0090]   Antisense approaches involve the design of nucleic acid molecules (e.g., DNA, RNA, or modified forms thereof) that are complementary to nucleic acids encoding a GRP. The antisense molecules will bind to GRP mRNA transcripts and prevent translation or to the endogenous gene and prevent transcription. Absolute complementarity is not required. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0091]   Antisense nucleic acid molecules that are complementary to the 5' end of an mRNA, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated region of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. (Wagner, R., Nature 372:333, 1994). Therefore, antisense molecules complementary to either the 5' or 3' untranslated, non-coding regions of a GRP mRNA or gene may be used in an antisense approach to inhibit transcription and/or translation of endogenous GRP gene or mRNA, respectively. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon.

[0092]   The coding strand sequences of GRPs are known. For example, Table 1 provides the coding sequences of some GRPs and related molecular chaperones. Other sequence will be readily apparent and available through GenBank.

Table 1

GRP	GenBank/NCBI Accession No.
GRP58	NM_005313 (SEQ ID NO:5 and 6)
GRP78	BC020235 and P11021 (SEQ ID NO:1 and 2)
GRP94	BC066656 and AAH66656 (SEQ ID NO:15 and 16)
Calreticulin (CALR)	NM_004343 and BT007448 (SEQ ID NO:7 and 8)
calreticulin 3 (CALR3)	NM_145046 (SEQ ID NO:9 and 10)
PDI	E03087 and NM_006849 (pancreatic) (SEQ ID NO:11 and 12)
ERp72	HUMERP72H (SEQ ID NO:13 and 14)

Each of the accession numbers and their content is incorporated herein by reference. Given the coding strand sequences encoding, for example, GRP78, antisense nucleic acids can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of a GRP polynucleotide (*e.g.*, GRP78 mRNA), or can be an oligonucleotide, which is antisense to only a portion of the coding or noncoding region of a GRP. For example, the antisense oligonucleotide can be complementary to the region surrounding the transcriptional or translation start site of GRP mRNA. An antisense oligonucleotide can be, for example, about 10, 20, 25, 50, 100, 150, 200, 250, 300, 350, 400 or more nucleotides in length. An antisense nucleic acid molecule can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. Antisense nucleic acid molecules of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al., Nucl. Acids Res. 16:3209, 1988; or methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451, 1988). An antisense nucleic acid molecule can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids.

[0093]   The invention includes antisense nucleic acid molecules, which hybridize with a polynucleotide sequence comprising a sequence encoding a GRP. The antisense molecules employed may be unmodified or modified RNA or DNA molecules. Suitable modifications include, but are not limited to, the ethyl or methyl phosphonate modification disclosed in U.S. Pat. No. 4,469,863, the disclosure of which is incorporated by reference, and the phosphorthioate modifications to deoxynucleotides described by LaPlanche, *et al.*, 1986 Nucleic Acids Research, 14:9081, and by Stec, *et al.*, 1984 J. Am. Chem Soc. 106:6077. The

modification to the antisense oligonucleotides is typically a terminal modification in the 5' or 3' region. Alternatively, the antisense molecules can have chimeric backbones of two or more modified nucleic acid bases, which are modified by different methods. Such methods include, for example, amino acid or nucleic acid modification as described by K. Ramasamy and W. Seifert (Bioorganic and Medicinal Chemistry Letters, 6(15):1799-1804 (1996)) or 4' sugar substituted olignucleotides described by G. Wang and W. Seifert (Tetrahedron Letters, 37(36):6515-6518 (1996)).

[0094]   Phosphodiester-linked oligonucleotides are particularly susceptible to the action of nucleases in serum or inside cells, and therefore in a one embodiment the antisense nucleic acid molecules of the invention are phosphorothioate or methyl phosphonate-linked analogues, which have been shown to be nuclease-resistant. Specific examples of some antisense oligonucleotides envisioned for this invention may contain phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar ("backbone") linkages. Typical are phosphorothioates and those with CH_2NHOCH_2, $CH_2N(CH_3)OCH_2$, $CH_2ON(CH_3)CH_2$, $CH_2N(CH_3)N(CH_3)CH_2$ and $ON(CH_3)CH_2CH_2$ backbones (where phosphodiester is $OPOCH_2$). Also typical are oligonucleotides having morpholino backbone structures (Summerton and Weller, U.S. Pat. No. 5,034,506). In other embodiments, 2'-methylribonucleotides (Inoue, et al., Nucleic Acids Research, 15:6131, 1987) and chimeric oligonucleotides that are composite RNA-DNA analogues (Inoue, et al., FEBS Lett., 215:327, 1987) may also be used for the purposes described herein. Finally, DNA analogues, such as peptide nucleic acids (PNA) are also included (Egholm, et al., Nature 365:566, 1993; Nielsen et al., Science, 254:1497, 1991) can be used according to the invention. Other oligonucleotides may contain alkyl and halogen-substituted sugar moieties comprising one of the following at the 2' position: OH, SH, SCH_3, F, OCN, OCH_3OCH_3, $OCH_3O(CH_2)nCH_3$, $O(CH_2)_nNH_2$ or $O(CH_2)_nCH_3$ where n is from 1 to about 10; C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF_3; OCF_3; O, S, or N-alkyl; O, S or N alkenyl; $SOCH_3$; SO_2CH_3; ONO_2; NO_2; N_3; NH_2;

heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a cholesteryl group; a conjugate; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of a oligonucleotide; or a group for improving the pharmacodynamic properties of a oligonucleotide and other substituents having similar properties. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group. Other embodiments may include at least one modified base form or "universal base" such as inosine. The preparation of base-modified nucleosides, and the synthesis of modified oligonucleotides using said base-modified nucleosides as precursors, has been described, for example, in U.S. Pat. Nos. 4,948,882 and 5,093,232. These base-modified nucleosides have been designed so that they can be incorporated by chemical synthesis into either terminal or internal positions of a oligonucleotide. Such base-modified nucleosides, present at either terminal or internal positions of a oligonucleotide, can serve as sites for attachment of a peptide or other antigen. Nucleosides modified in their sugar moiety have also been described (e.g., U.S. Pat. No. 5,118,802 and U.S. Pat. No. 5,681,940, both of which are incorporated by reference) and can be used similarly. Persons of ordinary skill in this art will be able to select other linkages for use in the invention. These modifications also may be designed to improve the cellular uptake and stability of the oligonucleotides. It is understood that depending on the route or form of administration of the antisense oligonucleotides of the invention, the modification or site of modification will vary (e.g., 5' or 3' modification). One of skill in the art can readily determine the appropriate modification without undue experimentation.

[0095]   Examples of modified nucleotides which can be used to generate the antisense molecules include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-

methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-
methyladenine, 2-methylguanine, 3-methylcytosine, 5-
methylcytosine, N6-adenine, 7-methylguanine, 5-
methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-
D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-
methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-
oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-
thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil,
5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-
oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-
carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.
Alternatively, the antisense molecule can be produced
biologically using an expression vector into which a GRP
polynucleotide or fragment thereof has been subcloned in an
antisense orientation (*i.e.*, RNA transcribed from the inserted
nucleic acid will be of an antisense orientation to a target
nucleic acid of interest).

[0096]   In a specific embodiment, the antisense molecule
comprises a sequence as set forth in SEQ ID NO:3 or a fragment
thereof. Thus, in one aspect, the antisense molecule includes
(i) SEQ ID NO:3; (ii) fragments of SEQ ID NO:3 which inhibit the
production of GRP78; (iii) SEQ ID NO:3 or a fragment thereof
wherein T is replaced with U; (iv) SEQ ID NO:3 having a modified
backbone; and (v) any of (i) - (iv) capable of interacting with a
polynucleotide encoding GRP78. Other specific antisense
molecules include antisense fragments of nucleic acids encoding
GRPs (*e.g.*, fragments of SEQ ID NO:3, antisense fragments of
GRP94 and the like; see Table 1).

[0097]   Small double stranded nucleic acid molecules that can
silence a GRP are also provided as part of the invention. Small
interfering RNA (siRNA) molecules are provided that interfere
with RNA transcription. RNA interference (RNAi) is a mechanism
of post-transcriptional gene silencing in which double-stranded
RNA (dsRNA) corresponding to a gene (or coding region) of
interest is introduced into a cell or an organism, resulting in
degradation of the corresponding mRNA. The RNAi effect persists
for multiple cell divisions before gene expression is regained.
RNAi is therefore an extremely powerful method for making

targeted knockouts or "knockdowns" at the RNA level. RNAi has
proven successful in human cells, including human embryonic
kidney and HeLa cells (see, *e.g.*, Elbashir *et al.*, Nature,
411(6836):494-8, 2001). In one embodiment, GRP (*e.g.*, GPR78)
silencing can be induced in mammalian cells by enforcing
endogenous expression of RNA hairpins (see Paddison *et al.*, PNAS
USA 99:1443-1448, 2002). In another embodiment, transfection of
small (21-23 nt) dsRNA specifically inhibits gene expression
(reviewed in Caplen, Trends in Biotechnology 20:49-51, 2002).

[0098]   Briefly, dsRNA corresponding to a portion of a GRP
gene to be silenced is introduced into a cell. The dsRNA can be
longer sequences that are subsequently digested into 21-23
nucleotide siRNAs, or short interfering RNAs, or the 21-23
nucleotide siRNA molecules may be directly provided to the cell.
The siRNA duplexes bind to a nuclease complex to form what is
known as the RNA-induced silencing complex, or RISC. The RISC
targets the homologous transcript by base pairing interactions
between one of the siRNA strands and the endogenous mRNA. It then
cleaves the mRNA about 12 nucleotides from the 3' terminus of the
siRNA (reviewed in Sharp *et al.*, Genes Dev 15: 485-490, 2001; and
Hammond *et al.*, Nature Rev Gen 2: 110-119, 2001).

[0099]   RNAi technology in gene silencing utilizes standard
molecular biology methods. dsRNA corresponding to the sequence
from a target gene to be inactivated can be produced by standard
methods, *e.g.*, by simultaneous transcription of both strands of a
template DNA (corresponding to the target sequence) with T7 RNA
polymerase. Kits for production of dsRNA for use in RNAi are
available commercially, *e.g.*, from New England Biolabs, Inc.
Methods of transfection of dsRNA or plasmids engineered to make
dsRNA are routine in the art.

[00100]   Gene silencing effects similar to those of RNAi have
been reported in mammalian cells with transfection of a mRNA-cDNA
hybrid construct (Lin *et al.*, Biochem Biophys Res Commun,
281(3):639-44, 2001), providing yet another strategy for gene
silencing.

[00101]   Accordingly, the invention provides small interfering
nucleic acids (siNA) that interact with a polynucleotide encoding
a GRP. In one aspect, the invention provides siNA comprising (i)

a sequence as set forth in SEQ ID NO:1, 5, 7, 9, 11, 13, or 15 as
set forth in Table 1, and their complement that is 21-23
nucleotides in length and comprises an AA dinucleotide at the 5'
end and a GC content of 30-50%; (ii) a double stranded nucleic
acid comprising 5'-AAGGTTACCCATGCAGTTGTT-3' (SEQ ID NO:4) and its
complement; (iii) a sequence as set forth in SEQ ID NO:4 and its
complement wherein T is replaced with U; and any of (i) - (iii)
wherein the siNA has a modified backbone as described above.

[00102]   Ribozyme molecules designed to catalytically cleave
GRP mRNA transcripts can also be used to prevent translation of
GRP mRNA and expression of GRP protein (see, e.g., PCT
Publication No. WO 90/11364, published Oct. 4, 1990; Sarver et
al., Science 247:1222-1225, 1990 and U.S. Pat. No. 5,093,246).
While ribozymes that cleave mRNA at site specific recognition
sequences can be used to destroy GRP mRNAs, the use of hammerhead
ribozymes is typical. Hammerhead ribozymes cleave mRNAs at
locations dictated by flanking regions that form complementary
base pairs with the target mRNA. The sole requirement is that the
target mRNA have the following sequence of two bases: 5'-UG-3'.
The construction and production of hammerhead ribozymes is well
known in the art and is described more fully in Haseloff and
Gerlach, Nature 334:585-591, 1988. Typically the ribozyme is
engineered so that the cleavage recognition site is located near
the 5' end of GRP mRNA; i.e., to increase efficiency and minimize
the intracellular accumulation of non-functional mRNA
transcripts. Ribozymes within the invention can be delivered to a
cell using a vector.

[00103]   Endogenous GRP gene expression can also be reduced by
inactivating or "knocking out" the GRP gene or its promoter using
targeted homologous recombination. See, e.g, Kempin et al.,
Nature 389: 802 (1997); Smithies et al., Nature 317:230-234,
1985; Thomas and Capecchi, Cell 51:503-512, 1987; and Thompson et
al., Cell 5:313-321, 1989. For example, a mutant, non-functional
GRP gene variant (or a completely unrelated DNA sequence) flanked
by DNA homologous to the endogenous GRP gene (either the coding
regions or regulatory regions of the GRP gene) can be used, with
or without a selectable marker and/or a negative selectable
marker, to transfect cells that express GRP protein in vivo.

[00104]   The nucleic acids, ribozyme, RNAi, and triple helix
molecules used in the invention may be prepared by any method
known in the art for the synthesis of DNA and RNA molecules.
These include techniques for chemically synthesizing
oligodeoxyribonucleotides and oligoribonucleotides well known in
the art such as for example solid phase phosphoramide chemical
synthesis. Alternatively, RNA molecules may be generated by *in
vitro* and *in vivo* transcription of DNA sequences encoding the
nucleic acid molecule. Such DNA sequences may be incorporated
into a wide variety of vectors, which incorporate suitable RNA
polymerase promoters. Alternatively, antisense cDNA constructs
that synthesize antisense RNA constitutively or inducibly,
depending on the promoter used, can be introduced stably into
cell lines.

[00105]   In yet another aspect, the invention provides
polypeptide antagonists of GRP activity. Such polypeptides
include antibodies, soluble domains of a GRP and polypeptides
that interact with a transmembrane domain of a GRP to prevent
incorporation of the GRP into a membrane of a cell. For example,
as described herein, GRP78 comprises a cytosolic domain and
transmembrane domains. The cytosolic domain (e.g., a soluble
domain) interacts with cytosolic proteins that induce apoptotis.
By inhibiting the interaction of the GRP with the cytosolic
proteins that induce apoptosis, the anti-apoptotic effect of the
GRP can be inhibited. Furthermore, GRPs (e.g., GRP78 comprises
hydrophobic transmembrane domain(s). For example, hydrophobic
transmembrane domain III (amino acids 210-260 of SEQ ID NO:2)
and/or domain IV (amino acids 400-450 of SEQ ID NO:2) of the
protein of GRP78 are useful targets. Polypeptide agents that
regulate the ability of a GRP polypeptide to integrate in to the
membrane are candidates for modulating apoptosis. For example, a
polypeptide agent that inhibits the ability of GRP78 to integrate
into the membrane will also be capable of promoting apoptosis
because it will prevent GRP78 from interacting with cytosolic
components that are required to promote apoptosis. Thus, variants
and fragments of a GRP protein (e.g., fragments, analogs and
derivatives of native GRP proteins) may also be used in methods

of the invention to inhibit anti-apoptotic activity of a GRP
(e.g., a GRP antagonist).

[00106]   As discussed above, the topology of GRP78 indicates
that part of GRP78 is exposed to the cytosol, allowing it to
interact with cytosolic components. Thus, in another embodiment,
the invention provides a method of modulating apoptosis by
contacting GRP78 with an agent that inhibits or prevents the
ability of the protein to integrate in to the membrane of the
endoplasmic reticulum. In yet another embodiment, the invention
provides a method of identifying an agent that modulates the
interaction of GRP78 with a membrane by providing a polypeptide
that includes hydrophobic transmembrane domain III (amino acids
210-260 of SEQ ID NO:2) and/or domain IV (amino acids 400-450 of
SEQ ID NO:2) of the protein of GRP78. The polypeptide can be
contacted with an agent, and the effect of the agent on the
interaction can be determined. Agents that regulate the ability
of the polypeptide to integrate in to the membrane are candidates
for modulating apoptosis. For example, an agent that inhibits
the ability of GRP78 to integrate in to the membrane will also be
capable of promoting apoptosis because it will prevent GRP78 from
interacting with cytosolic components that are required to
promote apoptosis. Accordingly, a method of the invention
includes modulating apoptosis by regulating the interaction
between ER membrane bound (i.e., integrally associated) GRP78 and
a cytosolic component that mediates apoptosis, such as, for·
example, a complex of proteins that includes caspase-7.

[00107]   In a further embodiment, the invention provides a
method of identifying an agent that modulates the interaction of
GRP78 with a cytosolic component that mediates apoptosis. The
method includes providing GRP78 integrally-associated with a
membrane, providing a cytosolic component comprising at least one
caspase, providing an agent, contacting the protein with the
component and the agent, simultaneously or in succession, and
determining the effect of the agent on the interaction of the
protein and the component as compared to a control.

[00108]   The invention provides methods and compositions that
are useful to promote apoptosis in a tissue or cell comprising
contacting the tissue or cell with an agent that inhibits the

anti-apoptotic activity of a GRP (e.g., GRP78). The methods and compositions are useful in treating neoplastic disorders including cancer and tumor growth. The methods and compositions can be used alone or in combination with other neoplastic/cancer therapies. For example, the methods and compositions of the invention can be used in combination with chemotherapeutic drugs such as, but not limited to, 5-fluorouracil (5FU), cytosine arabinoside, cyclophosphamide, cisplatin, carboplatin, doxyrubicin, etoposide, taxol, and alkylating agents. Furthermore, combinations of nucleic acid inhibitors may be used (e.g., a combination of SEQ ID NO:3 and SEQ ID NO:4).

[00109] In another aspect, variants and fragments of a GRP protein (e.g., fragments, analogs and derivatives of native GRP proteins) may also be used in methods of the invention. Such variants include, e.g., a polypeptide encoded by a naturally occurring allelic variant of a native GRP polynucleotide, a polypeptide encoded by an alternative splice form of a native GRP polynucleotide, a polypeptide encoded by a homolog of a native GRP polynucleotide, and a polypeptide encoded by a non-naturally occurring variant of a native GRP polynucleotide.

[00110] GRP protein variants have a polypeptide sequence that differs from a native GRP protein in one or more amino acids. The peptide sequence of such variants can feature a deletion, addition, or substitution of one or more amino acids of a native GRP polypeptide. Amino acid insertions are typically of about 1 to 4 contiguous amino acids, and deletions are preferably of about 1 to 10 contiguous amino acids. In some applications, variant GRP proteins substantially maintain a native GRP protein functional activity (e.g., ability to mediate anti-apoptotic activity, bind caspase-7 and the like; are agonists). Such functional variants are useful in treating disorders associated with apoptosis, e.g., ischemia and the like, where it is desirable to reduce apoptosis. For other applications, variant GRP proteins lack or feature a significant reduction in a GRP protein functional activity. Where it is desired to retain a functional activity of native GRP protein, a GRP protein variant can be made by expressing nucleic acid molecules that feature silent or conservative changes. Variant GRP proteins with

substantial changes in functional activity can be made by expressing nucleic acid molecules that feature less than conservative changes.

[00111]   GRP protein fragments corresponding to one or more particular motifs and/or domains or to arbitrary sizes, for example, at least 5, 10, 25, 50, 75, 100, 125, 150, or 175 amino acids in length may be utilized in methods of the invention. In addition, fragments can be chemically synthesized using techniques known in the art such as conventional solid phase f-Moc or t-Boc chemistry. For example, a GRP protein used in the methods of the invention may be arbitrarily divided into fragments of desired length with no overlap of the fragments, or divided into overlapping fragments of a desired length. The fragments can be produced (recombinantly or by chemical synthesis) and tested to identify those fragments, which can function as either agonists or antagonists of a native GRP protein.

[00112]   Methods of the invention may also involve recombinant forms of the GRP proteins. Recombinant polypeptides, in addition to native GRP protein, are encoded by a nucleic acid that has at least 85% sequence identity (e.g., 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%) with a native GRP nucleic acid sequence. In a one embodiment, variant GRP proteins lack one or more functional activities of a native GRP protein.

[00113]   GRP protein variants can be generated through various techniques known in the art. For example, GRP protein variants can be made by mutagenesis, such as by introducing discrete point mutation(s), or by truncation. Mutation can give rise to a GRP protein variant having substantially the same, or merely a subset of the functional activity of a native GRP protein. Alternatively, antagonistic forms of the protein can be generated which are able to inhibit the function of the naturally occurring form of the protein, such as by competitively binding to another molecule that interacts with GRP protein (e.g., interferes with the interaction of GRP78 and caspase-7). In addition, agonistic forms of the protein may be generated that constitutively express one or more GRP functional activities. Other variants of GRP proteins that can be generated include those that are resistant

to proteolytic cleavage, as for example, due to mutations that alter protease target sequences. Whether a change in the amino acid sequence of a peptide results in a GRP protein variant having one or more functional activities of a native GRP protein can be readily determined by testing the variant for a native GRP protein functional activity.

[00114]   Nucleic acid molecules encoding GRP fusion proteins may be used in methods of the invention. Such nucleic acids can be made by preparing a construct (e.g., an expression vector) that expresses a GRP fusion protein when introduced into a suitable host. For example, such a construct can be made by ligating a first polynucleotide encoding a GRP protein fused in frame with a second polynucleotide encoding another protein such that expression of the construct in a suitable expression system yields a fusion protein.

[00115]   As another example, GRP protein variants can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate polynucleotide sequence can be carried out in an automatic DNA synthesizer, and the synthetic polynucleotide then ligated into an appropriate expression vector. The purpose of a degenerate set of polynucleotides is to provide, in one mixture, all of the sequences encoding the desired set of potential GRP protein sequences. The synthesis of degenerate oligonucleotides is well known in the art (see for example, Narang, Tetrahedron 39:3, 1983; Itakura et al., Recombinant DNA, Proc 3rd Cleveland Sympos. Macromolecules, ed. A G Walton, Amsterdam: Elsevier pp 273-289, 1981; Itakura et al., Annu. Rev. Biochem. 53:323, 1984; Itakura et al., Science 198:1056, 1984; Ike et al., Nucleic Acid Res. 11:477, 1983. Such techniques have been employed in the directed evolution of other proteins (see, for example, Scott et al., Science 249:386-390, 1990; Roberts et al., Proc. Natl. Acad. Sci. USA 89:2429-2433, 1992; Devlin et al., Science 249: 404-406, 1990; Cwirla et al., Proc. Natl. Acad. Sci. USA 87: 6378-6382, 1990; as well as U.S. Pat. Nos. 5,223,409; 5,198,346; and 5,096,815).

[00116]   Similarly, a library of coding sequence fragments can be provided for a GRP clone in order to generate a variegated population of GRP polypeptide fragments for screening and

subsequent selection of fragments having one or more GRP agonist (e.g., anti-apoptotic) or antagonist (e.g., apoptotic) activities. A variety of techniques are known in the art for generating such libraries, including chemical synthesis. In one embodiment, a library of coding sequence fragments can be generated by (i) treating a double-stranded PCR fragment of a GRP polynucleotide coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule; (ii) denaturing the double-stranded DNA; (iii) renaturing the DNA to form double-stranded DNA which can include sense/antisense pairs from different nicked products; (iv) removing single-stranded portions from reformed duplexes by treatment with S1 nuclease; and (v) ligating the resulting fragment library into an expression vector. By this exemplary method, an expression library can be derived which codes for N-terminal, C-terminal and internal fragments of various sizes.

[00117]   A wide range of techniques are known in the art for screening products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of GRP polynucleotide variants. The most widely used techniques for screening large libraries typically involve cloning the library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. One screening technique useful to measure anti-apoptotic and apoptotic effects includes determining cell survival in the presence of etoposide. Recombinant products that inhibit (e.g., are antagonistic of) native GRP function will show an increase in cell-death in the presence of etoposide, whereas products that promote (e.g., are agonists of) GRP function will show a reduced cell-death compared to controls. Thus, the invention provides methods of mutagenizing and screening gene products to determine their agonistic and/or antagonistic effect on GRP activity. Agents

having agonistic effects are useful for treating ischemia and related disorders that cause unwanted cell death. Agents that have antagonistic effects are useful to treat diseases and disorders having unwanted cell growth (e.g., cell proliferative disorders associated with cancer and the like).

[00118]   Methods of the invention may utilize mimetics, e.g. peptide or non-peptide agents, that are able to disrupt binding of a GRP protein to other proteins or molecules with which a native GRP protein interacts (e.g., caspase-7). Thus, the mutagenic techniques described herein can also be used to map which determinants of GRP protein participate in the intermolecular interactions involved in, for example, binding of a GRP protein to other proteins which function to carry out apoptosis. Although the invention described thus far has focused on methods and compositions useful to promote apoptosis by inhibiting anti-apoptotic GRPs, the invention also includes methods and compositions that promote anti-apoptotic activity (e.g., in response ischemic injury and the like).

[00119]   The invention also provides methods and compositions, which promote anti-apoptotic activity of GRPs (e.g., GRP78 and 94). The compositions and methods of this aspect of the invention are useful to treat tissue damage or potential damage to cells or tissues resulting from, for example, stroke, heart attack, hypoxia, hypoglycemia, brain or spinal cord ischemia, or brain or spinal cord trauma. The methods use agents (including, e.g., small molecules, polypeptides, peptides, and nucleic acids) that promote GRP activity, GRP expression, GRP production, and/or GRP association with polypeptides resulting in an inhibition of apoptosis.

[00120]   The invention provides methods and compositions for modulating GRP expression and/or activity in a cell. Numerous agents for modulating expression/activity of intracellular proteins such as GRP in a cell are known. Any of these suitable for the particular system being used may be employed. Typical agents for promoting (e.g., agonistic) activity of GRPs include mutant/variant GRP polypeptides or fragments, nucleic acids encoding a functional GRP polypeptide or variant, and small organic or inorganic molecules.

[00121]   Examples of proteins that can modulate GRP expression and/or activity in a cell include native GRP proteins (*e.g.*, to upregulate activity) or variants thereof that can compete with a native GRP protein for binding ligands such as a caspase (*e.g.*, to downregulate apoptosis). Such protein variants can be generated through various techniques known in the art as described herein. For example, GRP protein variants can be made by mutagenesis, such as by introducing discrete point mutation(s), or by truncation (*e.g.*, of the transmembrane region). Mutation can give rise to a GRP variant or fragment having substantially the same, improved, or merely a subset of the functional activity of a native GRP protein. Agonistic (or superagonistic) forms of the protein may be generated that constitutively express one or more GRP functional activities. Other variants of GRP polypeptides that can be generated include those that are resistant to proteolytic cleavage, as for example, due to mutations which alter protease target sequences. Whether a change in the amino acid sequence of a peptide results in a GRP protein variant having one or more functional activities of a native GRP protein can be readily determined by testing the variant for a native GRP protein functional activity (*e.g.*, modulating apoptosis).

[00122]   As previously noted, the invention provides a method of inhibiting apoptosis in a tissue by overexpressing GRP78 and/or GRP94 in the targeted tissue. Overexpression of a polypeptide in a target tissue can be accomplished by any method known to the skilled artisan. For example, a nucleic acid sequence encoding GRP78 and/or GRP94 can be incorporated in a nucleic acid construct suitable for expression in a targeted tissue. Generally, the construct will possess the appropriate regulatory sequences for expression in the targeted tissue.

[00123]   The invention provides methods involving modulating levels of GRP in a cell. The cell may be *in vitro* or *in vivo*. Where the cell is *in vivo* it may be present in an animal subject such as any mammal including humans, rats, mice, cats, dogs, goats, sheep, horses, monkeys, apes, rabbits, cattle, and the like. The animal subject can be in any stage of development including adults, young animals, and neonates. Animal subjects

also include those in a fetal stage of development. Target
tissues can be any within the animal subject such as liver,
kidney, heart (e.g., cardiomyocytes), lungs, components of
gastrointestinal tract, pancreas, gall bladder, urinary bladder,
skeletal muscle, the central nervous system including the brain,
eye, skin, bones, and the like.

[00124]   Various techniques using viral vectors for the
introduction of a GRP nucleic acid (e.g., an inhibitory nucleic
acid such as an antisense molecule or a GRP variant) into a cell
may be utilized in the methods of the invention. Viral vectors
for use in the invention are those that exhibit low toxicity to a
host cell and induce production of therapeutically useful
quantities of a GRP protein or antisense and/or RNAi nucleic
acids in a tissue-specific manner. Viral vector methods and
protocols that may be used in the invention are reviewed in Kay
et al. Nature Medicine 7:33-40, 2001. The use of specific
vectors, including those based on adenoviruses, adeno-associated
viruses, herpes viruses, and retroviruses are described in more
detail below.

[00125]   The use of recombinant adenoviruses as gene therapy
vectors is discussed in W. C. Russell, Journal of General
Virology 81:2573-2604, 2000; and Bramson et al., Curr. Opin.
Biotechnol. 6:590-595, 1995. Adenovirus vectors are useful in the
invention because they (1) are capable of highly efficient gene
expression in target cells and (2) can accommodate a relatively
large amount of heterologous (non-viral) DNA. A typical form of
recombinant adenovirus is a "helper-dependent" adenovirus vector.
Such a vector features, for example, (1) the deletion of all or
most viral-coding sequences (those sequences encoding viral
proteins), (2) the viral inverted terminal repeats (ITRs) which
are sequences required for viral DNA replication, (3) up to 28-32
kb of "exogenous" or "heterologous" sequences (e.g., sequences
encoding a GRP protein, a GRP variant, an antisense molecule, or
an RNAi molecule), and (4) the viral DNA packaging sequence which
is required for packaging of the viral genomes into infectious
capsids

[00126]   Other viral vectors that might be used in the
invention are adeno-associated virus (AAV)-based vectors. AAV-

based vectors are advantageous because they exhibit high
transduction efficiency of target cells and can integrate into
the host genome in a site-specific manner. Use of recombinant AAV
vectors is discussed in detail in Tal, J., J. Biomed. Sci. 7:279-
291, 2000 and Monahan and Samulski, Gene Therapy 7:24-30, 2000. A
typical AAV vector comprises a pair of AAV inverted terminal
repeats (ITRs) which flank at least one cassette containing a
tissue (e.g., heart)- or cell (e.g., cardiomyocyte)-specific
promoter operably linked to a GRP nucleic acid. The DNA sequence
of the AAV vector, including the ITRs, the promoter and GRP gene
may be integrated into the host genome.

[00127]   The use of herpes simplex virus (HSV)-based vectors is
discussed in detail in Cotter and Robertson, Curr. Opin. Mol.
Ther. 1:633-644, 1999. HSV vectors deleted of one or more
immediate early genes (IE) are advantageous because they are
generally non-cytotoxic, persist in a state similar to latency in
the host cell, and afford efficient host cell transduction.
Recombinant HSV vectors can incorporate approximately 30 kb of
heterologous nucleic acid. A typical HSV vector is one that: (1)
is engineered from HSV type I, (2) has its IE genes deleted, and
(3) contains a tissue-specific promoter operably linked to a GRP
nucleic acid (e.g., an antisense, RNAi, GRP variant). HSV
amplicon vectors may also be useful in various methods of the
invention. Typically, HSV amplicon vectors are approximately 15
kb in length, and possess a viral origin of replication and
packaging sequences.

[00128]   Retroviruses such as C-type retroviruses and
lentiviruses are also useful in the invention. For example,
retroviral vectors may be based on murine leukemia virus (MLV).
See, e.g., Hu and Pathak, Pharmacol. Rev. 52:493-511, 2000 and
Fong et al., Crit. Rev. Ther. Drug Carrier Syst. 17:1-60, 2000.
MLV-based vectors may contain up to 8 kb of heterologous nucleic
acids in place of the viral genes. The heterologous nucleic acids
typically comprise a tissue-specific promoter and a GRP nucleic
acid.

[00129]   Additional retroviral vectors that might be used are
replication-defective lentivirus-based vectors, including human
immunodeficiency (HIV)-based vectors. See, e.g., Vigna and

Naldini, J. Gene Med. 5:308-316, 2000 and Miyoshi et al., J.
Virol. 72:8150-8157, 1998. Lentiviral vectors are advantageous in
that they are capable of infecting both actively dividing and
non-dividing cells. They are also highly efficient at transducing
human epithelial cells. Lentiviral vectors for use in the
invention may be derived from human and non-human (including SUV)
lentiviruses. A typical lentiviral vector includes nucleic acid
sequences required for vector propagation as well as a tissue-
specific promoter operably linked to a GRP nucleic acid.

[00130]   A lentiviral vector may be packaged into any suitable
lentiviral capsid. The substitution of one particle protein with
another from a different virus is referred to as "pseudotyping".
The vector capsid may contain viral envelope proteins from other
viruses, including murine leukemia virus (MLV) or vesicular
stomatitis virus (VSV). The use of the VSV G-protein yields a
high vector titer and results in greater stability of the vector
virus particles.

[00131]   Alphavirus-based vectors, such as those made from
semliki forest virus (SFV) and sindbis virus (SIN), might also be
used in the invention. Use of alphaviruses is described in
Lundstrom, K., Intervirology 43:247-257, 2000 and Perri et al.,
Journal of Virology 74:9802-9807, 2000. Alphavirus vectors
typically are constructed in a format known as a replicon. A
replicon may contain (1) alphavirus genetic elements required for
RNA replication, and (2) a heterologous nucleic acid such as one
encoding a GRP nucleic acid.

[00132]   Recombinant, replication-defective alphavirus vectors
are advantageous because they are capable of high-level gene
expression, and can infect a wide host cell range. Alphavirus
replicons may be targeted to specific cell types by displaying on
their virion surface a functional ligand or binding domain that
would allow selective binding to target cells expressing a
cognate binding partner. Alphavirus replicons may establish
latency, and therefore long-term heterologous nucleic acid
expression in a host cell. The replicons may also exhibit
transient heterologous nucleic acid expression in the host cell.
To increase tissue selectivity of the virus and reduce risk not
only can such a virus have a targeted ligand on the virion

surface, but also the heterologous nucleic acid (*e.g.*, a GRP nucleic acid) can be operably linked to a tissue specific promoter.

[00133]   In addition to viral vector-based methods, non-viral methods may also be used to introduce a GRP nucleic acid into a host cell. A review of non-viral methods of gene delivery is provided in Nishikawa and Huang, Human Gene Ther. 12:861-870, 2001. A non-viral gene delivery method according to the invention employs plasmid DNA to introduce a GRP nucleic acid into a cell. Plasmid-based gene delivery methods are generally known in the art and are described in references such as Ilan, Y., Curr. Opin. Mol. Ther. 1:116-120, 1999, Wolff, J. A., Neuromuscular Disord. 7:314-318, 1997 and Arztl, Z., Fortbild Qualitatssich 92:681-683, 1998.

[00134]   Methods involving physical techniques for introducing a GRP nucleic acid into a host cell can be adapted for use in the invention. For example, the particle bombardment method of gene transfer utilizes an Accell device (gene gun) to accelerate DNA-coated microscopic gold particles into a target tissue, *e.g.*, a cancer tissue. See, *e.g.*, Yang *et al.*, Mol. Med. Today 2:476-481 1996 and Davidson *et al.*, Rev. Wound Repair Regen. 6:452-459, 2000. As another example, cell electropermeabilization (also termed cell electroporation) may be employed to deliver GRP nucleic acids into cells. See, *e.g.*, Preat, V., Ann. Pharm. Fr. 59:239-244 2001.

[00135]   Synthetic gene transfer molecules can be designed to form multimolecular aggregates with plasmid DNA. These aggregates can be designed to bind to a target cell surface in a manner that triggers endocytosis and endosomal membrane disruption. Cationic amphiphiles, including lipopolyamines and cationic lipids, may be used to provide receptor-independent GRP nucleic acid transfer into target cells. In addition, preformed cationic liposomes or cationic lipids may be mixed with plasmid DNA to generate cell-transfecting complexes. Methods involving cationic lipid formulations are reviewed in Felgner *et al.*, Ann. N.Y. Acad. Sci. 772:126-139, 1995 and Lasic and Templeton, Adv. Drug Delivery Rev. 20:221-266, 1996. For gene delivery, DNA may also be coupled

to an amphipathic cationic peptide (Fominaya et al., J. Gene Med. 2:455-464, 2000).

[00136]   DNA microencapsulation may be used to facilitate delivery of a GRP nucleic acid. Microencapsulated gene delivery vehicles may be constructed from low viscosity polymer solutions that are forced to phase invert into fragmented spherical polymer particles when added to appropriate nonsolvents. Methods involving microparticles are discussed in Hsu et al., J. Drug Target 7:313-323, 1999 and Capan et al., Pharm. Res. 16:509-513, 1999.

[00137]   Protein transduction offers an alternative to gene therapy for the delivery of therapeutic proteins into target cells, and methods involving protein transduction are within the scope of the invention. Protein transduction is the internalization of proteins into a host cell from the external environment. The internalization process relies on a protein or peptide which is able to penetrate the cell membrane. To confer this ability on a normally non-transducing protein, the non-transducing protein can be fused to a transduction-mediating protein such as the antennapedia peptide, the HIV TAT protein transduction domain, or the herpes simplex virus VP22 protein. See Ford et al., Gene Ther. 8:1-4, 2001.

[00138]   There are two major approaches to getting the nucleic acid (optionally contained in a vector) into a subject's cells; in vivo and ex vivo. For in vivo delivery the nucleic acid is injected directly into the subject, usually at the site where the nucleic acid is needed. For ex vivo treatment, the subject's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the subject either directly or, for example, encapsulated within porous membranes which are implanted into the subject (see, e.g., U.S. Pat. Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells in vitro, or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, microinjection, cell

fusion, DEAE-dextran, the calcium phosphate precipitation method, viral vectors and the like. A commonly used vector for *ex vivo* and in vivo delivery is a viral vector as discussed above.

[00139] Host cells can be transfected or transformed with expression or cloning vectors described herein and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the nucleic acids encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*

[00140] Methods of eukaryotic cell transfection and prokaryotic cell transformation are known to the ordinarily skilled artisan, for example, $CaCl_2$, $CaPO_4$, liposome-mediated and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes. Infection with Agrobacterium tumefaciens is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 Jun. 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transfections have been described in U.S. Pat. No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, Methods in Enzymology, 185:527-537 (1990) and Mansour *et al.*, Nature, 336:348-352 (1988).

[00141]   Suitable host cells for cloning or expressing the DNA
in the vectors herein include prokaryote, yeast, or higher
eukaryote cells. Suitable prokaryotes include but are not limited
to eubacteria, such as Gram-negative or Gram-positive organisms,
for example, Enterobacteriaceae such as *E. coli*. Various *E. coli*
strains are publicly available, such as *E. coli* K12 strain MM294
(ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110
(ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable
prokaryotic host cells include Enterobacter, Erwinia, Klebsiella,
Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia,
e.g., Serratia marcescans, and Shigella, as well as Bacilli such
as B. subtilis and B. licheniformis (e.g., B. licheniformis 41P
disclosed in DD 266,710 published 12 Apr. 1989), Pseudomonas such
as P. aeruginosa, and Streptomyces. These examples are
illustrative rather than limiting.

[00142]   In addition to prokaryotes, eukaryotic microbes such
as filamentous fungi or yeast are suitable cloning or expression
hosts. *Saccharomyces cerevisiae* is a commonly used lower
eukaryotic host microorganism. Others include *Schizosaccharomyces
pombe*, Kluyveromyces hosts such as, e.g., *K. lactis*, *K. fragilis*,
K. bulgaricus, *K. wickeramii*, *K. waltii*, *K. drosophilarum*, *K.
thermotolerans*, and *K. marxianus*; yarrowia; *Pichia pastoris*;
Candida; *Trichoderma reesia*; *Neurospora crassa*; *Schwanniomyces*
such as *Schwanniomyces occidentalis*; and filamentous fungi such
as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus*
hosts such as *A. nidulans* and *A. niger*. Methylotropic yeasts are
suitable herein and include, but are not limited to, yeast
capable of growth on methanol selected from the genera consisting
of Hansenula, Candida, Kloeckera, Pichia, Saccharomyces,
Torulopsis, and Rhodotorula. A list of specific species that are
exemplary of this class of yeasts may be found in C. Anthony, The
Biochemistry of Methylotrophs, 269 (1982).

[00143]   Examples of useful mammalian host cell lines are
monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL
1651); human embryonic kidney line (293 or 293 cells subcloned
for growth in suspension culture, Graham et al., J. Gen Virol.
36:59,1977); baby hamster kidney cells (BHK, ATCC CCL 10);
Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc.

Natl. Acad. Sci. USA 77:4216, 1980); mouse sertoli cells (TM4,
Mather, Biol. Reprod. 23:243-251, 1980); monkey kidney cells (CV1
ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC
CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2);
canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells
(BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75);
human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT
060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad.
Sci. 383:44-68, 1982); MRC 5 cells; FS4 cells; and a human
hepatoma line (Hep G2).

[00144]   Host cells are transformed with the above-described
GRP nucleic acid expression or cloning vectors and cultured in
conventional nutrient media modified as appropriate for inducing
promoters, selecting transformants, or amplifying the nucleic
acids encoding the desired sequences.

[00145]   The GRP nucleic acids (*e.g.*, antisense, RNAi,
ribozymes, variants, coding sequences and the like) may be
inserted into a replicable vector for cloning (amplification of
the DNA) or for expression. Various vectors are publicly
available. The vector may, for example, be in the form of a
plasmid, cosmid, viral particle, or phage. The appropriate
nucleic acid sequence may be inserted into the vector by a
variety of procedures. In general, DNA is inserted into an
appropriate restriction endonuclease site(s) using techniques
known in the art. Vector components generally include, but are
not limited to, one or more of a signal sequence, an origin of
replication, one or more marker genes, an enhancer element, a
promoter, and a transcription termination sequence. Construction
of suitable vectors containing one or more of these components
employs standard ligation techniques which are known to the
skilled artisan.

[00146]   Expression and cloning vectors will typically contain
a selection gene, also termed a selectable marker. Typical
selection genes encode proteins that (a) confer resistance to
antibiotics or other toxins, *e.g.*, ampicillin, neomycin,
methotrexate, or tetracycline, (b) complement auxotrophic
deficiencies, or (c) supply critical nutrients not available from

complex media, e.g., the gene encoding D-alanine racemase for Bacilli.

[00147]   An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the BDB oligopeptide-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub et al., Proc. Natl. Acad. Sci. USA, 77:4216, 1980. A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb et al., Nature, 282:39, 1979; Kingsman et al., Gene, 7:141, 1979; Tschemper et al., Gene, 10:157, 1980). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan.

[00148]   A variety of well-known techniques can be used to identify polypeptides which specifically bind to, for example, GRP78 and/or caspase-7, and regulate their interactions. Exemplary techniques include mobility shift DNA-binding assays, methylation and uracil interference assays, DNase and hydroxy radical footprinting analysis, fluorescence polarization, and UV crosslinking or chemical cross-linkers. For a general overview, see, e.g., Ausubel (chapter 12, DNA-Protein Interactions). Furthermore, biological assays the measure the agonistic and antagonistic effects of such agents are also provided.

[00149]   For example, the invention provides a screening assay to determine the GRP agonistic or antagonistic effect an agent may have on a cell. The assay comprises contacting a cell expressing a GRP with an agent suspected to have GRP agonist or antagonist activity. Contacting the cell with a chemotherapeutic agent (e.g., etoposide) and measuring the percent survival of the cell or cells in culture. Where cell survival is increased compared to a control, the agent has agonistic activity, wherein the cell survival is decreased the agent has agonistic activity.

[00150]   In another embodiment, the invention provides pharmaceutical compositions comprising an agent identified by a method of the invention, and instructions for use of the agent in the treatment of a cell proliferative disorder. For example, an agent identified as regulating the interaction between GRP78 and

a cytosolic component, or an agent that regulates the ability of
GRP78 to integrate in to a membrane, such as an ER membrane, can
be included in a pharmaceutical composition to treat a cell
proliferative disorder. The treatment can encompass inhibiting
the disorder by promoting apoptosis or by inhibiting apoptosis.
For example, the methods of the invention are suitable for use in
preventing dividing cells from further replication by promoting
apoptosis or in preventing non-dividing cells from destruction by
inhibiting apoptosis.

[00151]   The invention provides methods and compositions for
treating a subject having a cell proliferative disorder. The
subject can be any mammal, and is preferably a human. The
contacting can be in vivo or ex vivo. Methods of administering
pharmaceutical compositions are known in the art and include, for
example, systemic administration, topical administration,
intraperitoneal administration, intra-muscular administration, as
well as administration directly at the site of a tumor or cell-
proliferative disorder and other routes of administration known
in the art.

[00152]   The pharmaceutical compositions according to the
invention may be administered locally or systemically. By
"therapeutically effective dose" is meant the quantity of a
compound according to the invention necessary to prevent, to cure
or at least partially arrest the symptoms of the disease and its
complications. Amounts effective for this use will, of course,
depend on the severity of the disease and the weight and general
state of the subject. Typically, dosages used in vitro may
provide useful guidance in the amounts useful for in situ
administration of the pharmaceutical composition, and animal
models may be used to determine effective dosages for treatment
of particular disorders. Various considerations are described,
e.g., in Langer, Science, 249: 1527, (1990); Gilman et al. (eds.)
(1990), each of which is herein incorporated by reference.

[00153]   As used herein, "administering a therapeutically
effective amount" is intended to include methods of giving or
applying a pharmaceutical composition of the invention to a
subject that allow the composition to perform its intended
therapeutic function. The therapeutically effective amounts will

vary according to factors such as the degree of infection in a subject, the age, sex, and weight of the individual. Dosage regimen can be adjusted to provide the optimum therapeutic response. For example, several divided doses can be administered daily or the dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation.

[00154]   As used herein, a "pharmaceutically acceptable carrier" is intended to include solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the pharmaceutical composition, use thereof in the therapeutic compositions and methods of treatment is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00155]   The principal pharmaceutical composition is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in an acceptable dosage unit. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

[00156]   Further, methods of the invention can be performed alone or in conjunction with standard medical treatments currently available for treating a cell proliferative disorder. For example, when a tumor is being treated, it may be preferable to remove the majority of a tumor surgically or by radiation prior to introducing a construct of the invention in to the cells comprising the tumor.

[00157]   The terms "protein", "peptide" and "polypeptide" as used herein, describe any chain of amino acids, regardless of length or post-translational modification (for example, glycosylation or phosphorylation). Thus, the terms can be used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid. Thus, the term

"polypeptide" includes full-length, naturally occurring proteins as well as recombinantly or synthetically produced polypeptides that correspond to a full-length naturally occurring protein or to particular domains or portions of a naturally occurring protein. The term also encompasses mature proteins which have an added amino-terminal methionine to facilitate expression in prokaryotic cells.

[00158]   Polypeptides and peptides can be chemically synthesized using known techniques or produced using known molecular biology techniques. Polypeptides and proteins are encoded in the genome of an organism by nucleic acids in discrete functional units sometimes referred to as "genes". Nucleic acid molecules, however, can be removed and isolated from their naturally occurring environment and engineered and manipulated using molecular biology techniques. The term "isolated" means altered "by the hand of man" from its natural state; i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring nucleic acid molecule or a polypeptide naturally present in a living animal in its natural state is not "isolated", but the same nucleic acid or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

[00159]   "Polynucleotide" or "nucleic acid molecule" refers to a polymeric form of nucleotides at least 10 bases in length. By "isolated nucleic acid" is meant a polynucleotide that is not immediately contiguous with either of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an automatically replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA) independent of other sequences. The nucleic acid molecules of the invention may comprise ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double stranded forms.

[00160]   The term nucleic acid molecule(s) or polynucleotide(s) generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as used herein refers to, among others, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions.

[00161]   In addition, a polynucleotide or nucleic acid molecule as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide.

[00162]   As used herein, the term polynucleotide includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "nucleic acid molecules" as that term is intended herein.

[00163]   Nucleic acid molecules comprising an antisense molecule, a siRNA molecule, or encoding a GRP polypeptide and the like, as disclosed herein, can be operatively linked to expression control element(s). "Operatively linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. An expression control element(s) operatively linked to a nucleic acid molecule of the invention is ligated such that transcription of the nucleic acid molecule is achieved under conditions compatible with the expression control element(s). As used herein, the term "expression control element(s)" refers to control domain that regulate the expression of a nucleic acid molecule to which it is operatively linked. Expression control element(s) are operatively linked to a nucleic acid molecules

when the expression control element(s) control and regulate the
transcription and, as appropriate, translation of the nucleic
acid molecule. Thus, expression control element(s) can include
appropriate promoters, enhancers, transcription terminators, a
start condon (i.e., ATG) in front of a protein-encoding nucleic
acid, splicing signals for introns, maintenance of the correct
reading frame of that gene to permit proper translation of the
mRNA, and stop condons. The term "control element(s)" is intended
to include, at a minimum, components whose presence can influence
expression, and can also include additional components whose
presence is advantageous, for example, leader sequences and
fusion partner sequences. Expression control element(s) can
include a promoter.

[00164]   By "promoter" is meant a minimal nucleic acid domain
sufficient to direct transcription. Also included in the
invention are those promoter elements which are sufficient to
render promoter-dependent gene expression controllable for cell-
type specific, tissue-specific, or inducible by external signals
or agents; such elements may be located in the 5′ or 3′ regions
of the gene. Both constitutive and inducible promoters, are
included in the invention (see e.g., Bitter et al., Methods in
Enzymology 153:516-544, 1987). For example, when cloning in
bacterial systems, inducible promoters such as pL of
bacteriophage-γ, plac, ptrp, ptac (ptrp-lac hybrid promoter) and
the like may be used. When cloning in mammalian cell systems,
promoters derived from the genome of mammalian cells (e.g.,
metallothionein promoter) or from mammalian viruses (e.g., the
retrovirus long terminal repeat; the adenovirus late promoter;
the vaccinia virus 7.5K promoter) may be used. Promoters produced
by recombinant DNA or synthetic techniques may also be used to
provide for transcription of the nucleic acids of the invention.

[00165]   A nucleic acid molecule may be designed to selectively
hybridize to a target polynucleotide or oligonucleotide under
desired conditions. The phrase "selectively (or specifically)
hybridizes to" refers to the binding, duplexing, or hybridizing
of a molecule only to a particular nucleotide sequence under
stringent hybridization conditions when that sequence is present
in a complex mixture.

[00166]   The phrase "stringent hybridization conditions" refers
to conditions under which a nucleic acid molecule will hybridize
to its target complementary sequence, typically in a complex
mixture of nucleic acids, but to no other sequences. In the
context of the invention, stringent conditions comprises
hybridization and washing under which nucleotide sequences at
least 60% homologous to each other typically remain hybridized to
each other. Typically, the conditions are such that sequences at
least about 65%-70% or 75% or more homologous to each other
typically remain hybridized to each other.

[00167]   Generally, stringent conditions are selected to be
about 5 to 10 °C lower than the thermal melting point (T_m) for the
specific sequence at a defined ionic strength pH. The T_m is the
temperature (under defined ionic strength, pH, and nucleic acid
concentration) at which 50% of the nucleic acid molecules
complementary to the target hybridize to the target sequence at
equilibrium (as the target sequences are present in excess, at T_m,
50% of the probes are occupied at equilibrium). Stringent
conditions will be those in which the salt concentration is less
than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium
ion concentration (or other salts) at pH 7.0 to 8.3 and the
temperature is at least about 30 °C for short probes (for
example, 10 to 50 nucleotides) and at least about 60 °C. for long
probes (for example, greater than 50 nucleotides). Stringent
conditions also may be achieved with the addition of
destabilizing agents, for example, formamide.

[00168]   Exemplary highly stringent hybridization conditions
can be as following, for example: 50% formamide, 5xSSC and 1%
SDS, incubating at 42 °C, or 5xSSC and 1% SDS, incubating at 65
°C, with wash in 0.2xSSC and 0.1% SDS at 65 °C. Alternative
conditions include, for example, conditions at least as stringent
as hybridization at 68 °C for 20 hours, followed by washing in
2xSSC, 0.1% SDS, twice for 30 minutes at 55 °C and three times
for 15 minutes at 60 °C. Another alternative set of conditions is
hybridization in 6xSSC at about 45 °C, followed by one or more
washes in 0.2xSSC, 0.1% SDS at 50-65 °C. Exemplary moderately
stringent hybridization conditions include a hybridization in a

buffer of 40% formamide, 1 M NaCl, 1% SDS at 37 °C, and a wash in 1xSSC at 45 °C.

[00169]   "Treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. A subject or mammal is successfully "treated" for a neoplastic disorder/cancer if, after receiving a therapeutic amount of a GRP antagonist the subject shows observable and/or measurable reduction in or absence of one or more of the following: reduction in the number of cancer cells or absence of the cancer cells; reduction in the tumor size; inhibition (i.e., slow to some extent and preferably stop) of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition (i.e., slow to some extent and preferably stop) of tumor metastasis; inhibition, to some extent, of tumor growth; and/or relief to some extent, one or more of the symptoms associated with the specific cancer; reduced morbidity and mortality, and improvement in quality of life issues.

[00170]   The phrase "non-dividing" cell refers to a cell that does not go through mitosis. Non-dividing cells may be blocked at any point in the cell cycle, (e.g., G0/G1, G1/S, G2/M), as long as the cell is not actively dividing. Examples of pre-existing non-dividing cells in the body include neuronal, muscle, liver, skin, heart, lung, and bone marrow cells, and their derivatives.

[00171]   By "dividing" cell is meant a cell that undergoes active mitosis, or meiosis. Such dividing cells include stem cells, skin cells (e.g., fibroblasts and keratinocytes), gametes, and other dividing cells known in the art. Of particular interest and encompassed by the term dividing cell are cells having cell proliferative disorders, such as neoplastic cells. The term "cell proliferative disorder" refers to a condition characterized by an abnormal number of cells. The condition can include both hypertrophic (the continual multiplication of cells

resulting in an overgrowth of a cell population within a tissue) and hypotrophic (a lack or deficiency of cells within a tissue) cell growth or an excessive influx or migration of cells into an area of a body. The cell populations are not necessarily transformed, tumorigenic or malignant cells, but can include normal cells as well.

[00172]   Cell proliferative disorders include disorders associated with an overgrowth of connective tissues, such as various fibrotic conditions, including scleroderma, arthritis and liver cirrhosis. Cell proliferative disorders include neoplastic disorders such as head and neck carcinomas. Head and neck carcinomas would include, for example, carcinoma of the mouth, esophagus, throat, larynx, thyroid gland, tongue, lips, salivary glands, nose, paranasal sinuses, nasopharynx, superior nasal vault and sinus tumors, esthesioneuroblastoma, squamous call cancer, malignant melanoma, sinonasal undifferentiated carcinoma (SNUC) or blood neoplasia. Also included are carcinoma's of the regional lymph nodes including cervical lymph nodes, prelaryngeal lymph nodes, pulmonary juxtaesophageal lymph nodes and submandibular lymph nodes (Harrison's Principles of Internal Medicine (eds., Isselbacher, et al., McGraw-Hill, Inc., 13th Edition, pp1850-1853, 1994). Other cancer types, include, but are not limited to, lung cancer, colon-rectum cancer, breast cancer, prostate cancer, urinary tract cancer, uterine cancer lymphoma, oral cancer, pancreatic cancer, leukemia, melanoma, stomach cancer and ovarian cancer.

[00173]   Methods involving conventional molecular biology techniques are described herein. Such techniques are generally known in the art and are described in detail in methodology treatises such as Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; and Current Protocols in Molecular Biology, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates). Various techniques using polymerase chain reaction (PCR) are described, e.g., in Innis et al., PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, 1990. PCR-primer pairs can be derived from known sequences by known

techniques such as using computer programs intended for that purpose (e.g., Primer, Version 0.5, 81991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Methods for chemical synthesis of nucleic acids are discussed, for example, in Beaucage and Carruthers, Tetra. Letts. 22:1859-1862, 1981, and Matteucci et al., J. Am. Chem. Soc. 103:3185, 1981. Chemical synthesis of nucleic acids can be performed, for example, on commercial automated oligonucleotide synthesizers. Immunological methods (e.g., preparation of antigen-specific antibodies, immunoprecipitation, and immunoblotting) are described, e.g., in Current Protocols in Immunology, ed. Coligan et al., John Wiley & Sons, New York, 1991; and Methods of Immunological Analysis, ed. Masseyeff et al., John Wiley & Sons, New York, 1992. Conventional methods of gene transfer and gene therapy can also be adapted for use in the invention. See, e.g., Gene Therapy: Principles and Applications, ed. T. Blackenstein, Springer Verlag, 1999; Gene Therapy Protocols (Methods in Molecular Medicine), ed. P. D. Robbins, Humana Press, 1997; and Retro-vectors for Human Gene Therapy, ed. C. P. Hodgson, Springer Verlag, 1996.

[00174]   A few diseases and disorders (e.g., ischemia and neoplastic disorders) have been mentioned. However, those of skill in the art will recognize that a variety of diseases and degenerative disorders involve aberrant or disregulated apoptosis, resulting in inappropriate or premature cell death or inappropriate cell proliferation. For example, inhibition of cell death may contribute to disease in the immune system by allowing the persistence of self-reactive B and T cells, which leads to autoimmune disease. Furthermore, the infection by certain viruses may depend on suppression of host cell death by anti-apoptotic viral gene products and inhibition of apoptosis can alter the course (lytic vs. latent) of viral infection.

EXAMPLES

[00175]   The invention is based, in part, on the discovery that GRPs (e.g., GRP78) confers resistance to topoisomerase inhibitors through protection against drug-induced apoptosis. As shown in Figure 1, panel A, quantitation of the immunoblots of whole cell extracts showed 5-fold higher GRP78 level in C.1 cells compared with the parental CHO cells, whereas the level of GRP94, also an

ER-localized chaperone protein, and a 45-kDa unidentified protein (X) recognizable by the anti-KDEL antibody was relatively constant in both cell lines. *In situ* immunofluorescence imaging using anti-GRP78 antibody further revealed that in both CHO and C.1 cells, the majority of GRP78 was concentrated in the perinuclear region, consistent with its location in the ER (Figure 1, Panel B). The intensity of the immunofluorescent signal for GRP78 was greater in the majority of C.1 cells compared with CHO cells.

[00176] In examining the distribution of GRP78 and caspase-7 *in situ* using immunofluorescence, caspase-7 exhibits a perinuclear pattern indicative of ER localization (Figure 1, Panel C). Confocal microscopy further revealed caspase-7 is in close proximity with a subfraction of GRP78. The co-localization of GRP78 and caspase-7 was primarily detected in the perinuclear/ER region.

[00177] The physical interaction of endogenous caspase-7 with GRP78 was further confirmed using whole cell extracts prepared from CHO and C.1 cells. In agreement with the co-localization results obtained from confocal microscopy, procaspase-7 forms a complex with a high level of GRP78 in C.1 cells (Figure 6, Panel A, lane 2). For CHO cells, GRP78 was detected as a faint band in the anti-caspase-7 immunoprecipitate using an anti-KDEL antibody (Figure 6, Panel A, lane 1), and the signal for GRP78 was very enhanced when an anti-hamster GRP78 antibody was used for the Western blots (Figure 6, Panel B, lane 1). Using anti-caspase-3 as the immunoprecipitating antibody, GRP78 was not detected associated with procaspase-3 in Western blots (Figure 6, Panel A, lanes 3 and 4). Thus, endogenous GRP78 constitutively associates with procaspase-7. While the data provided herein indicates that GRP78 and caspase-7 interact, the invention is not limited to a direct interaction between the two proteins. It is understood that the invention encompasses a cytosolic component that is a complex of polypeptides, including caspase-7, or caspase-7 individually. By preventing the interaction of GRP78 with caspase, the agent modulates apoptosis by promoting apoptosis. Alternatively, by promoting the interaction of GRP78 with

caspase-7, an agent would modulate apoptosis by inhibiting apoptosis.

[00178]   As shown in Figure 7, Panel A, at low dose of trypsin digestion, a resistant carboxyl band of about 35kDa was detected. At the higher dose of trypsin, the intensity of the 35-kDa band became stronger, and a minor band of around 50-kDa was also visible.   The digestion pattern for the CHO cells was the same, with the resistant bands more prominent for C.1 cells correlating with GRP78 overexpression.   The trypsin treatment did not digest ER proteins localized inside the ER lumen as confirmed by the calreticulin control (Figure 7, Panel B).

[00179]   In addition, sodium carbonate extraction of the microsome membrane fractions indicates that GRP78 is located in both the membrane and lumenal (Figure 7, Panel C).   These results show that GRP78 is not exclusively an ER lumen protein, rather a subpopulation exist as a transmembrane protein.   This is consistent with domains III and IV serving as putative transmembrane domains, with carboxyl fragments locating inside the lumen of the ER rendering them resistant to trypsin digestion (Figure 7, Panel A).   This topology further indicates that part of GRP78 is exposed to the cytosol, allowing it to interact with cytosolic components.   Thus, in another embodiment, the invention provides a method of modulating apoptosis by contacting glucose regulated protein 78 (GRP78) with an agent that inhibits or prevents the ability of the protein to integrate in to the membrane of the endoplasmic reticulum.   In yet another embodiment, the invention provides a method of identifying an agent that modulates the interaction of glucose regulated protein 78 (GRP78) with a membrane by providing a polypeptide that includes hydrophobic transmembrane domain III (amino acids 210-260 of SEQ ID NO:1 or 2) and/or domain IV (amino acids 400-450 of SEQ ID NO:1 or 2) of the protein of glucose regulated protein 78. The polypeptide can be contacted with an agent, and the effect of the agent on the interaction can be determined. Agents that regulate the ability of the polypeptide to integrate in to the membrane are candidates for modulating apoptosis.   For example, an agent that inhibits the ability of GRP78 to integrate in to the membrane will also be capable of promoting apoptosis because

it will prevent GRP78 from interacting with cytosolic components that are required to promote apoptosis.

[00180]   It was further determined that the ATP-binding domain of GRP78 is necessary for the interaction with cytosolic components.   The ATP binding domain resides in the amino portion of GRP78 (Figure 6, Panel C).   The invention further encompasses the use of fragments of GRP78 containing the ATP-binding domain in the methods of the invention.   For example, a polypeptide that includes amino acids 125-275 of SEQ ID NO:1 or 2, or amino acids 150-250 of SEQ ID NO:1 or 2, or amino acids 175-201 of SEQ ID NO:1 or 2 can be used in a method to identify an agent that regulates the interaction of GRP78 and cytosolic components.

[00181]   CHO cell line AD-1 that stably expresses a deleted form of GRP78 (Figure 6, Panel C) was used to determine that the ATP-binding region of GRP78 is necessary for binding to caspase. The deletion spans residues 175 to 201 within the ATP binding domain, resulting in defective ATPase activity.   Western blot analysis of whole cell extracts prepared from CHO and AD-1 cells confirmed expression of the deleted GRP78 form, in addition to endogenous wild-type GRP78, in AD-1 cells (Fig. 6B, lanes 3 and 4).

[00182]   Immunoprecipitation using anti-caspase-7 antibody showed that whereas procaspase-7 is able to form a complex with wild-type GRP78, deletion of residues 175 to 201 abolished its ability to bind to procaspase-7 (Figure 6, Panel B, lanes 1 and 2.   Upon etoposide treatment, AD-1 cells showed more caspase-7 activation *in vivo* and more extensive DNA fragmentation compared with C.1 cells that overexpress the wild-type protein (Figure 8, Panels A and B).   Annexin labeling and clonogenic survival assays performed with AD-1 cells further showed no protection against etoposide treatment compared with the parental CHO cells.

[00183]   A transient transfection cell death assay further indicated that inhibition of apoptosis by GRP78 was dependent upon the GRP78-caspase interaction.   Cell viability was measured quantitatively by the retention of β-galactosidase activity in the cells after drug treatment.   As shown in Figure 8, cells transfected with the expression vector for wild-type GRP78 conferred protection against etoposide treatment.   To confirm

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that the ATP binding function of GRP78 is required for the
protective effect of GRP78, the cells were transfected with
vectors expressing either wild-type GRP78 or mutant GRP78
(G227D), which carries an amino acid substitution at position
227, destroying its ATP binding ability. As shown in Figure 8,
Panel C, the protective effect was lost with the mutant form of
GRP78.

[00184] Experiments were performed to look at the effect of
GRP78 on chemotherapeutics. A strong cellular promoter (CMV) was
used to drive expression of GRP78 in the context of a well-
characterized adenovirus vector called pShuttle-CMV. Both the
sense and antisense orientation of GRP78 were constructed, to
serve the function of overexpression or suppression of GRP78. Two
versions of adenovirus with CMV promoter driving antisense (AS)
Grp78 were constructed (see Figures 11A-C). The construction
scheme for the full length AS construct is shown in Figure 11. An
adenovirus construct expressing a partial length AS comprising a
320 bp fragment of the grp78 exon I was cloned in reverse
orientation to the CMV promoter (see Fig. 11C). This shorter
fragment targets the AUG start codon and may be more effective
than the full length antisense molecule.

[00185] Overexpression of the His-tagged GRP78 in a human 293T
tissue culture test system was performed. This cell line was used
because it can be infected very efficiently with adenovirus. For
this purpose, different doses of adenovirus expressing His-tagged
GRP78 was infected into 293T cells. After 72 hr, the cell lysate
was prepared and immunoblot was performed to detect the level of
His-tagged GRP78, using antibody against the His-tagged which is
specific for the adenovirus expressed protein. The results showed
that His-tagged GRP78 was expressed in high levels in a dosage
dependent manner (Figure 12, lanes 4, 5 and 6). This proves that
the adenovirus construct for expressing full length GRP78 is
successful. The next step is to repeat these experiments in MDA
and MCF-7 cells. This step is more difficult because the cells
are harder to culture and they are more difficult to infect.

[00186] As a proof of principle, the two antisense (AS)
constructs were tested to determine their ability to suppress the
His-tagged GRP78 in the human 293T tissue culture test system.

For this purpose, different doses of adenovirus expressing either the full length AS or 320 bp AS was co-infected with adenovirus expressing His-tagged GRP78 into 293T cells. After 72 hr, the cell lysate was prepared and immunoblot was performed to detect the level of His-tagged GRP78, using antibody against the His-tagged which is specific for the adenovirus expressed protein. The results showed that both the full length AS (Figure 12, lanes 7, 8 and 9) and the 320 bp AS (Figure 12, lanes 1, 2 and 3) were able to suppress expression of the His-tagged GRP78 in a dosage dependent manner. This proves that the adenovirus constructs for expressing antisense GRP78 are successful. The most drastic reduction was obtained with the 320 bp AS (Figure 12, lane 1).

[00187] Next human breast cancer MDA-MB-435 cells were infected with the adenovirus expressing the 320 bp AS, in the presence or absence of treatment with the chemotherapy drug etoposide. The same cells were infected with the GFP negative control adenovirus. The results showed that in the mock-infected cells, etoposide treatment by itself reduced the amount of endogenous GRP78 by about 40% (Figure 13, lanes 1 and 2). Importantly, the 320 bp AS construct further reduced GRP78 level significantly, particularly in cells treated with etoposide, such that the final level was less than 10% (Figure 13, lanes 3 and 4). Thus, both the full length and the 320 bp version of the AS construct targeted against GRP78 blocked expression of GRP78 in a 293T test system. This was repeated in human breast cancer cells and showed that the AS adenovirus is able to suppress endogenous GRP78 expression.

[00188] Experiments were also performed to determine the anti-apoptotic effect conferred by overexpression of GRP78. This task was completed. GRP78 overexpression confers resistance to all four drugs (cisplatin, doxorubicin, etoposide and camptothecin). The results are summarized in Table 2.

Table 2

	% Survival (Colony Assay)	
	Control	GRP78 Overexpression
Cisplatin (µM)		
0	100	100
3.3	43	60
6.6	3.7	50
9.9	0	27
13.0	0	16

Doxorubicin (µg/ml)		
0	1	100
0.2	80	95
0.4	42	84
0.6	2	68
0.8	0	48
1.0	0	32
Etoposide (µM)		
0	100	100
0.2	71	93
0.4	67	93
0.8	26	77
1.6	1.5	63
Camptothecin (ng/ml)		
0	100	100
5	68	82
20	0.7	22
60	0	2

[00189]   The antisense approach described above was replaced
with an siRNA approach. The sequence of the Grp78 siRNA included:

5'   AAGGTTACCCATGCAGTTGTT 3'   (SEQ ID NO:4)

3'   TTCCAATGGGTACGTCAACAA 5' (SEQ ID NO:17)

When blasted against the human genomic sequence, this sequence is
unique and in principle, should not affect any other human gene.
To prove this, experiments were performed to test the effect of
this siRNA on the expression of GRP78 and related stress proteins
GRP94 and HSP70 in human 293T cells. The level of β-actin was
used as loading control. As shown in Figure 14, only GRP78 level
is suppressed, confirming that the siRNA is specific for GRP78.

[00190]   Experiments further showed that Grp78(II) siRNA at 80
nM or higher can significantly suppress GRP78 level in human
breast cancer MDA-MB-435 cells (Figure 15). This translates to
more death in the cells treated with Grp78(II)siRNA than control
siRNA targeted against the unrelated green fluorescence protein
(Figure 16).

[00191]   One candidate target of GRP78 action is BIK, the BH3-
only protein inducible in response to DNA damage that is located
in the ER as well as the mitochondria. Remarkably, ER-targeted
BIK can induce cytochrome c release, suggesting it can act at the
ER site to initiate a parallel cell death pathway (Germain et
al., 2002). To test whether GRP78, as a molecular chaperone, can
either directly or indirectly block the action of pro-apoptotic
molecules that control the release of cytochrome c from the
mitochondria the following experiment was performed. Using 293T

as a model system, BIK level was observed to increase following
etoposide treatment and co-IP of GRP78 with endogenous BIK
(Figure 17A). These results suggesting that GRP78 overexpression
can protect cells from cell death mediated by ER-targeted BIK
(Figure 17B). These findings imply that in cells treated with
etoposide, there are both physical and functional interactions
between BIK and GPP78. Thus, this can contribute in part to the
protective effect of GRP78 towards etoposide-induced cell death.

[00192]   Cell Culture Conditions — The CHO cells were
maintained in α-minimum Eagle's medium with nucleosides
supplemented with 10% dialyzed fetal calf serum and 1%
penicillin/streptomycin/neomycin antibiotics. The C.1 and AD-1
cells were maintained in the above conditions in the presence of
0.1 μg/ml methotrexate but without added nucleosides. The
establishment of stable T24/83 human transitional bladder
carcinoma cell lines overexpressing human GRP78 or transfected
with the empty expression vector (pcDNA3.1) has been described.
The T24/83 cell lines were maintained in M199 medium supplemented
with 10% fetal calf serum containing 1%
penicillin/streptomycin/neomycin antibiotics and 200 μg/ml G418.
The human acute T cell leukemia Jurkat cells were maintained in
RPMI 1640 medium supplemented with 10% fetal calf serum
containing 1% penicillin/streptomycin/neomycin antibiotics. All
the cells were maintained at 37°C in a humidified atmosphere of
5% CO_2/95% air.

[00193]   Reagents — Etoposide (Calbiochem) was dissolved in
Me_2SO at a concentration of 30 mM and stored at -20 °C.
Methotrexate (Sigma) was dissolved in a minimum amount of 1 M
NaOH, diluted with water to 1μg/ml, and stored at -20°C.
Doxorubicin (Bedford Laboratories, Bedford, OH) at 2 mg/ml and
camptothecin (Amersham Biosciences) at 20mg/ml were supplied as
isotonic solutions.

[00194]   Cell Cycle Analysis — Following seeding, exponentially
growing cells were trypsinized at different days and fixed in 70%
ethanol. The fixed cells were treated with PBS containing 0.1%
(v/v) Triton X-100, 0.2 mg/ml DNase-free RNase, and 20 μg/ml
propidium iodide (PI) for 30 min at room temperature. The cell
cycle distributions were analyzed by fluorescence-activated cell

sorting (FACS) analysis (FACstar; BD Biosciences). The cell
cycle distribution measurements were repeated three to four
times.

[00195]   Clonogenic Survival Assays — Four thousand cells were
seeded into 10-cm-diameter dishes. Two days after seeding, cells
were treated with etoposide for 6 h, doxorubicin for 1 h, or
camptothecin for 24 h at different concentrations as indicated.
After drug treatment, the cells were grown in fresh medium for 10
to 14 days. The colonies were washed with ice-cold PBS, fixed
with methanol, and stained with 10% Giemsa staining solution. The
surviving fraction was determined by dividing the number of the
surviving colonies in the drug-treated cells by the number of
colonies in the non-treated control groups.

[00196]   Annexin V Staining and FACS Analysis — CHO, C.1, and
T24/83 cells were trypsinized, washed twice with ice-cold PBS, pH
7.4, and resuspended in 1μ binding buffer (10 mM HEPES, pH 7.4,
140 mM NaCl, 2.5 mM $CaCl_2$ at a concentration of 1 x 10^6 cells/ml.
One hundred μl of cell suspension was transferred to 5-ml plastic
tubes, and 5 μl of annexin V-fluorescein isothiocyanate
(PharMingen) and 4 μl of 0.5 mg/ml PI were added. The cells were
gently vortexed and incubated in the dark at room temperature for
20 min. Four hundred μl of binding buffer was added to each
tube, and annexin V staining was analyzed by flow cytometry
within 1 h. Cells negative for both PI and annexin V staining
are live cells, annexin V positive staining cells are early
apoptotic cells, and PI positive and annexin V positive staining
cells are primarily cells in late stages of apoptosis.

[00197]   Caspase-7 Activation Assays — The cells were either
non-treated or treated with 100 μM etoposide for 6 h and
harvested after 24 h. The cells were suspended in 5 volumes of a
hypotonic buffer (5 mM Tris-HCl, pH 7.4, 5 mM KCl, 1.5 mM MgCl2,
0.1 mM EGTA, pH 8.0, and 1 mM dithiothreitol) in the presence of
2 μg/ml leupeptin, pepstatin, and aprotinin protease inhibitors.
After incubation on ice for 20 min, sucrose was added to a final
concentration of 250 mM, and the cells were disrupted by douncing
eight times in a 1-ml Wheaton Dounce homogenizer. The homogenate
was centrifuged twice at 750 μ g for 10 min. The supernatant was
clarified again at 16,000 x g for 15 min at 4 °C and designated

as the cytoplasmic fraction. For *in vitro* caspase-7 activation assay, 150 µg of cell-free extract was incubated with various amounts of cytochrome c and dATP at 37 °C for 1 h. Equal amounts of total proteins were separated, and Western blotting was performed for caspase-7.

[00198] Western Blotting – The cell lysate was prepared in radioimmune precipitation assay buffer and subjected to immunoblot with antibodies against GRP78, GRP94, topoisomerase II, caspase-7, and α-actin as described. Nitrocellulose membranes containing the transferred proteins were blocked in Tris-buffered saline containing 5% non-fat dry milk and 0.1% Tween 20 for 1 h at room temperature and were probed with the respective primary antibodies. For GRP78, an anti-KDEL mouse monoclonal antibody (SPA-827), an anti-GRP78 rabbit polyclonal antibody directed against the carboxyl ten amino acids of rat GRP78 (SPA-826) (StressGen, Victoria, Canada), or an anti-hamster GRP78 rabbit polyclonal antibody at 1:3000, 1:2000, and 1:5000 dilution, respectively, was used. Dilutions for the other primary antibodies were as follows: anti-calnexin rabbit polyclonal antibody (SPA-865) (StressGen) at 1:2000, anti-calreticulin rabbit polyclonal antibody (SPA-600) (StressGen) at 1:3000, anti-β-actin mouse monoclonal antibody (Sigma) at 1:5000, anti-caspase-7 mouse monoclonal antibody (10-1-62) (BD Biosciences) at 1:1000, anti-caspase-3 rabbit polyclonal antibody (Cell Signaling, Beverly, MA) at 1:1000, and anti-topoisomerase II mouse monoclonal antibody (SWT3D1) (Oncogene, San Diego, CA) at 1:1000. Respective horseradish peroxidase-conjugated secondary antibodies were used, and the protein bands were visualized by the ECL method (Amersham Biosciences).

[00199] Transient Transfection Death Assay – Briefly, Jurkat cells were transiently transfected with either CMV-neo-Bcl2 or expression vectors for wild-type hamster GRP78 or a GRP78 ATP-binding site mutant G227D. After drug treatment, cell lysates were prepared and assayed for β-galactosidase activity remaining in the surviving cells. The percent cytotoxicity was calculated as described previously.

[00200] DNA Fragmentation Assays – The cells were either non-treated or treated with 100 µM etoposide for 12 h and harvested

after 48 h.   The DNA fragmentation assays were performed using an apoptosis DNA ladder kit (Roche Molecular Biochemicals) according to manufacturer's instructions.

[00201]   Immunofluorescence Staining and Image Analysis — CHO and C.1 cells were grown to 60% confluence in chamber slides (Nalge Nunc International, Naperville, IL), washed twice with PBS, and fixed with 4% paraformaldehyde in PBS for 10 min. The cells were then washed with PBS and permeabilized in PBS containing 0.1% Triton X-100 and 5% bovine serum albumin for 30 min.   For detection of GRP78, the cells were stained with a 1:1000 dilution of anti-GRP78 (C-20) goat polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and a 1:500 dilution of anti-goat Texas red-conjugated secondary antibody (Vector Labs, Burlingame, CA).   For detection of caspase-7, the cells were stained with anti-caspase-7 mouse monoclonal antibody (BD Biosciences) at a 1:500 dilution and a 1:500 dilution of anti-mouse fluorescein isothiocyanate-conjugated secondary antibody (Vector Labs).   Cells were mounted in Vectashield with DAPI mounting medium (Vector Labs) and visualized on a Zeiss LSM 510 dual-photon confocal microscope.   The T24/83 cells were incubated with the same anti-GRP78 polyclonal antibody as described for the CHO cells. Whole cell images were subsequently captured and analyzed using Northern exposure image analysis/archival software (Mississauga, Ontario, Canada).

[00202]   Co-immunoprecipitation Assays — 2×10^6 cells were lysed in 400 µl of extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Nonidet P-40, and 0.5% deoxycholate, with protease inhibitor tablet (Roche Molecular Biochemicals)) and frozen and thawed three times.   500 µg of total protein extract from each sample was pretreated with 50 µl of protein A-Sepharose beads (Sigma) for 1 h at 4 °C prior to incubation with 5 µg of either anti-caspase-7 mouse monoclonal antibody or anticaspase-3 antibodies for 2 h. Following the incubation period, 50 µl of protein A-Sepharose beads was added, and the mixtures were rotated at 4 °C overnight. The beads were then washed five times with the extraction buffer.   The immunoprecipitate was released from the washed beads by the addition of 30 µl of 1x SDS-PAGE sample loading buffer (50 mM Tris-HCl, pH 6.8, 100 mM

dithiothreitol, 2% SDS, 0.1% bromphenol blue, 10% glycerol),
followed by heating at 100 °C for 10 min. The supernatant
obtained after centrifugation was resolved by SDS-PAGE and
subjected to Western blot analysis to detect the co-
immunoprecipitated proteins.

[00203]   Isolation of Microsomes and Protease Digestion — The
cells were trypsinized, and after washing with cold PBS, were
lysed by incubation in 10 volumes of cold hypotonic buffer (10 mM
Tris-HCl, pH 7.4), followed by Dounce homogenization. The lysate
was immediately adjusted to 0.25 M sucrose, 1 mM MgCl2 and
centrifuged at 1000 x g for 10 min at 4 °C to remove nuclei and
cell debris. The supernatant was further centrifuged at 100,000 x
g for 90 min. The pellet, representing microsomes, was rinsed
briefly with cold water and resuspended in 50 mM Tris-HCl, pH
7.4, and used for proteolytic digestion and sodium-carbonated
extraction studies.

[00204]   Sodium Carbonate Extraction—For separation of ER
membranes from lumenal proteins, the microsome pellet was
resuspended in 50 volumes of 100 mM sodium carbonate, pH 11.5,
and incubated on ice for 1 h. The suspension was then centrifuged
for 1 h at 240,000 x g at 4 °C. The pellet, which represents ER
membrane, was rinsed with cold water and resuspended in 1x SDS-
PAGE sample loading buffer and analyzed by Western blot.
Proteins present in the ER lumen were recovered from the
supernatant by the addition of trichloroacetic acid to a final
concentration of 10%. The pellet was washed three times with
acetone, air-dried, solubilized in the 1x SDS-PAGE sample loading
buffer, and analyzed by Western blot.

[00205]   Limited Tryptic Digestion of Microsomal Proteins — For
trypsin digestion reactions, the microsomes were incubated with
trypsin (0.01% or 0.05%) for 30 min at room temperature. The
proteolytic cleavage reactions were terminated by the addition of
1x SDS-PAGE sample loading buffer and boiling at 100 °C for 5
min. 10-20 µg of total protein from each reaction was analyzed
by Western blot.

[00206]   To examine directly whether specific overexpression of
GRP78 can lead to the development of drug resistance, CHO and C.1
cells were exposed to various drugs, and cell survival was

measured using clonogenic survival assays. Various dosages of
etoposide (also referred to as VP16), adriamycin (also referred
to as doxorubicin), and camptothecin were tested. Both etoposide
and adriamycin are inhibitors of topoisomerase II, and
camptothecin is a topoisomerase I inhibitor. The results for
each of the drugs tested are shown in Figure 2. With all three
drugs, C.1 cells overexpressing GRP78 conferred higher resistance
than CHO cells. These data establish that specific
overexpression of GRP78, in the absence of the UPR, is sufficient
to render CHO cells more resistant to topoisomerase I and II
inhibitors.

[00207]   To determine whether GRP78 protects the cells from
etoposide-induced apoptosis, CHO and C.1 cells were either
nontreated or treated with etoposide and labeled with annexin V
and PI. The apoptotic cells were identified by annexin V
labeling. For CHO cells, the percentage of apoptotic cells
increased 10-fold (from 9 to 90%) upon etoposide treatment; for
C.1 cells, the increase was 4.7-fold (from 15 to 70%) (Fig. 3A).
More extensive DNA fragmentation was also detected in etoposide-
treated CHO but not C.1 cells (Figure, Panel B).

[00208]   A pair of stably transfected human transitional
bladder carcinoma T24/83 cell lines selected and cultured under
identical conditions, were used to determine the effect of GRP
overexpression on neoplastic cells. The cell line, referred to
as T24/83-GRP78, overexpressed human GRP78, and the other line,
referred to as T24/83-pcDNA, was stably transfected with the
empty expression vector pcDNA (28). Immunoblot analysis followed
by normalization against α-actin revealed a 3-fold increase in
the level of GRP78 expression in the T24/83-GRP78 cells as
compared with T24/83-pcDNA cells (Fig. 4A, inset).
Overexpression of GRP78 in T24/83-GRP78 cells did not affect the
expression level of ER chaperone proteins GRP94, protein
disulfide isomerase and calreticulin, or heat shock protein HSP47
(Figure 4, Panel A). Whole cell imaging revealed much greater
GRP78 immunofluorescence for the T24/83-GRP78 cells, confirming
the results of the immunoblots (Figure 4, Panel B).

[00209]   In agreement with the CHO cell lines, T24/83 cells
overexpressing GRP78 exhibited more resistance to etoposide in

clonogenic survival assays (Figure, 4, Panel A). Similar protection was observed for adriamycin and camptothecin. For T24/83-cDNA cells, etoposide treatment increased the percentage of annexin V-labeled cells 2.7-fold (from 7 to 19%), as compared with an increase of 1.4-fold (from 8 and 11%) for T24/83-GRP78 cells (Fig. 4C).

[00210]   With the availability of the GRP78 overexpressing cell lines, the effect of GRP78 overexpression on topoisomerase II level in the absence of an UPR was determined. CHO and C.1 cells were either non-treated or treated with etoposide, and the level of topoisomerase II was determined by immunoblotting (Figure 5, Panel A). The data indicate that pecific GRP78 overexpression has no effect on the topoisomerase II protein level.

[00211]   Analysis of the cell cycle distribution of exponentially growing cells showed CHO and C.1 cells with similar G1, S, and G2 distribution profiles (Figure 9). In contrast, CHO cells treated with tunicamycin or thapsigargin, both standard UPR inducers, showed more cells in G1 and a dramatic reduction in S phase cells. A similar pattern was observed for exponentially growing T24/83 cells. In both the vector-transfected and GRP78 overexpressing cells, the percentage of G1, S, and G2 cells is similar. Cells treated with tunicamycin or thapsigargin showed a higher percentage of G1 cells and a lower percentage in S phase (Figure 9). Collectively, these results show that in contrast to the UPR, specific overexpression of GRP78 does not alter the cell cycle distribution.

[00212]   A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1.   A method of modulating apoptosis in a cell, the method
      comprising contacting a glucose regulated protein (GRP) with
      an agent that regulates the interaction of the GRP with a
      cytosolic component that mediates apoptosis.

2.   The method of claim 1, wherein the GRP is located in the
      endoplasmic reticulum.

3.   The method of claim 1, wherein the GRP is GRP94 or GRP78.

4.   The method of claim 1, wherein the GRP is GRP78.

5.   The method of claim 1, wherein the cytosolic component that
      mediates apoptosis is a caspase.

6.   The method claim 5, wherein the caspase is selected from the
      group consisting of Ced-3, caspase-1, caspase-2, caspase-4,
      caspase-5, caspase-6, caspase-7, caspase-8, caspase-9,
      caspase-10, and caspase 11-14.

7.   The method of claim 6, wherein the caspase is caspase-7.

8.   The method of claim 1, wherein the cytosolic component is a
      complex of polypeptides.

9.   The method of claim 1, wherein the modulating is by
      inhibiting apoptosis.

10.   The method of claim 9, wherein the method comprises
      contacting the cell with a nucleic acid encoding a GRP
      protein or functional fragment thereof.

11.   The method of claim 10, wherein the GRP protein is GRP78.

12.   The method of claim 10, wherein the nucleic acid is
      contained in an expression vector.

13.   The method of claim 10, wherein the nucleic acid is operably
      linked to a control element.

14.   The method of claim 13, wherein the control element is a
      promoter.

15.   The method of claim 10, wherein the GRP78 fragment comprises
      an soluble fragment of GRP78 that interacts with the
      cytosolic component.

16.   The method of claim 1, wherein the modulating is by
      promoting apoptosis.

17. The method of claim 16, wherein the modulating is by inhibiting the interaction of the GRP with the cytosolic component or inhibiting the production of GRP.

18. The method of claim 17, wherein the agent is a small molecule, a protein, a peptide, a peptidomimetic, a nucleic acid molecule or a combination thereof.

19. The method of claim 18, wherein the polypeptide is an antibody.

20. The method of claim 18, wherein the agent is a small molecule.

21. The method of claim 20, wherein the agent is versielostatin (VST), dATP or a combination thereof.

22. The method of claim 20, wherein the agent interacts with the ATP-binding domain of the protein.

23. The method of claim 22, wherein the agent is ATP, or derivative thereof, that binds to the ATP-binding domain of the protein.

24. The method of claim 23, wherein the ATP-binding domain comprises amino acids 125-275 of SEQ ID NO:2.

25. The method of claim 22, wherein the agent interacts with amino acids 150-250 of SEQ ID NO:2.

26. The method of claim 22, wherein the agent interacts with amino acids 175-201 of SEQ ID NO:2.

27. The method of claim 18, wherein the nucleic acid is an antisense molecule, a ribozyme, a siRNA, or a combination thereof.

28. The method of claim 27, wherein the antisense molecule interacts with a GRP78 polynucleotide.

29. The method of claim 27, wherein the siRNA interacts with a GRP78 polynucleotide.

30. The method of claim 28, wherein the antisense molecule comprises a sequence that is at least 80% identical to SEQ ID NO:1.

31. The method of claim 30, wherein the antisense molecule comprises a sequence that is at least 90% identical to SEQ ID NO:1.

32. The method of claim 30, wherein the antisense molecule comprises SEQ ID NO:3.

33. The method of claim 30, wherein the antisense molecule consists of SEQ ID NO:3.

34. The method of claim 29, wherein the siRNA comprises a sequence that is about 90% identical to SEQ ID NO:4 and its complement.

35. The method of claim 33, wherein the siRNA comprise a sequence that is about 95% identical to SEQ ID NO:4 and its complement.

36. The method of claim 29, wherein the siRNA comprises SEQ ID NO:4 and its complement.

37. The method of claim 28, wherein the siRNA consists of SEQ ID NO:4 and its complement.

38. The method of claim 27, wherein the nucleic acid is delivered to the cell in a vector.

39. The method of claim 1, wherein the cell is contacted *in vitro*.

40. The method of claim 1, wherein the cell is contact *in vivo*.

41. The method of claim 9, wherein the apoptosis results from a disease or disorder associated with stroke, heart attack, hypoxia, hypoglycemia, brain or spinal cord ischemia, or brain or spinal cord trauma.

42. The method of claim 1, wherein the modulating is promoting apoptosis and the cell is a neoplastic cell.

43. The method of claim 16, wherein the cell is a neoplastic cell.

44. A method of promoting apoptosis in a cell, the method comprising inhibiting glucose regulated protein 78 (GRP78) with an agent that (i) inhibits or prevents the ability of GRP78 to interact with a cytosolic protein and/or (ii) inhibits the production of GRP78.

45. The method of claim 44, wherein the agent is a polypeptide.

46. The method of claim 45, wherein the polypeptide is an antibody.

47. The method of claim 44, wherein the agent is a small molecule.

48. The method of claim 44, wherein the agent interacts with a hydrophobic transmembrane domain III (amino acids 210-260 of SEQ ID NO:1 or 2) or domain IV (amino acids 400-450 of SEQ ID NO:1 or 2) of the protein.

49. A method of identifying an agent that modulates the interaction of glucose regulated protein 78 (GRP78) with a cytosolic component that mediates apoptosis, the method comprising:

a) providing glucose regulated protein 78 (GRP78) integrally-associated with a membrane;

b) providing a cytosolic component comprising at least one caspase;

c) providing an agent;

d) contacting the protein of a) with the component of b) and the agent of c) simultaneously or in succession; and

e) determining the effect of the agent on the interaction of the protein and the component as compared to a control.

50. The method of claim 49, wherein the caspase is caspase-7.

51. The method of claim 49, wherein the glucose regulated protein 78 (GRP78) integrally-associated with a membrane is a microsome complex.

52. The method of claim 49, wherein the effect of the agent is to inhibit the interaction of the protein and the component.

53. The method of claim 49, wherein the assay is performed on a cell.

54. The method of claim 53, further comprising contacting the cell with etoposide.

55. The method of claim 54, wherein the determining is by measuring cell survival in the presence and absence of the agent.

56. A glucose regulated protein (GRP) inhibitory nucleic acid molecule comprising a nucleic acid that interacts with a glucose regulated protein (GRP) polynucleotide.

57. The GRP inhibitory nucleic acid molecule of claim 56, wherein the nucleic acid is an antisense molecule.

58. The antisense molecule of claim 57, wherein the antisense molecule is selected from the group consisting of:

(a)   a nucleic acid comprising a sequence that is at least
80% identical to SEQ ID NO:3 or a fragment and specifically
interacts with a GRP polynucleotide;

(b) a nucleic acid comprising a sequence that is at least
90% identical to SEQ ID NO:3 or a fragment and specifically
interacts with a GRP polynucleotide;

(c)   a nucleic acid comprising a sequence that is at least
95% identical to SEQ ID NO:3 or a fragment and specifically
interacts with a GRP polynucleotide;

(d)   a nucleic acid comprising a sequence as set forth in
SEQ ID NO:3; and

(e)   a nucleic acid consisting of the sequence as set forth
in SEQ ID NO:3.

59.   The antisense molecule of claim 56, wherein the GRP
      polynucleotide is a GRP78 polynucleotide.

60.   The GRP inhibitory nucleic acid molecule of claim 56,
      wherein the nucleic acid is a small inhibitory nucleic acid
      (siNA) molecule.

61.   The GRP inhibitory nucleic acid molecule of claim 60,
      wherein the siNA molecule is selected from the group
      consisting of:

(a)   a nucleic acid comprising a sequence that is at least
90% identical to SEQ ID NO:4 and its complement and
specifically interacts with a GRP polynucleotide;

(b)   a nucleic acid comprising a sequence that is at least
95% identical to SEQ ID NO:4 and its complement and
specifically interacts with a GRP polynucleotide;

(c)   a nucleic acid comprising a sequence as set forth in
SEQ ID NO:4 and its complement; and

(d) a nucleic acid consisting of the sequence set forth in
SEQ ID NO:4 and its complement.

62.   A glucose regulated protein modulating agent comprising a
      soluble domain of a GRP protein.

63.   A method of identifying an agent that modulates the
      interaction of glucose regulated protein 78 (GRP78) with a
      cytosolic component that mediates apoptosis, the method
      comprising:

(a)   providing a polypeptide comprising the ATP-binding
domain of glucose regulated protein 78 (GRP78);

(b).   providing a cytosolic component comprising at least
one caspase;

(c)   providing an agent;

(d)   contacting the polypeptide of a) with the component of

(e) and the agent of c) simultaneously or in succession; and

(f)   determining the effect of the agent on the interaction
of the polypeptide and the component as compared to a
control.

64.   The method of claim 63, wherein the polypeptide comprises
amino acids 125-275 of SEQ ID NO:2.

65.   The method of claim 63, wherein the polypeptide comprises
amino acids 150-250 of SEQ ID NO:2.

66.   The method of claim 63, wherein the polypeptide comprises
amino acids 175-201 of SEQ ID NO:1 or 2.

67.   A method of identifying an agent that modulates the
interaction of glucose regulated protein 78 (GRP78) with a
membrane, the method comprising:

(a)   providing a polypeptide comprising the hydrophobic
transmembrane domain III (amino acids 210-260 of SEQ ID NO:1
or 2) and/or domain IV (amino acids 400-450 of SEQ ID NO:1
or 2) of the protein of glucose regulated protein 78
(GRP78);

(b)   providing an agent;

(c)   contacting the polypeptide of a) the agent of b)
simultaneously or in succession; and

(d)   determining the effect of the agent on the interaction
of the polypeptide with the membrane as compared to a
control.

68.   A method of modulating apoptosis, the method comprising
contacting a cell comprising a caspase polypeptide with an
agent that regulates the interaction of the polypeptide with
glucose regulated protein 78 (GRP78) endoplasmic reticulum
transmembrane protein.

69.   The method claim 68, wherein the caspase is selected from
the group consisting of Ced-3, caspase-1, caspase-2,

caspase-4, caspase-5, caspase-6, caspase-7, caspase-8,
caspase-9, caspase-10, and caspase 11-14.

70.   The method of claim 69, wherein the caspase is caspase-7.

71.   The method of claim 68, wherein the modulating is by
promoting apoptosis.

72.   The method of claim 68, wherein the modulating is by
inhibiting apoptosis.

73.   The method of claim 68, wherein the agent is a polypeptide.

74.   The method of claim 68, wherein the agent is a small
molecule.

75.   The method of claim 74, wherein the polypeptide is a
fragment of the polypeptide consisting of SEQ ID NO:2.

76.   The method of claim 75, wherein the fragment comprises a
soluble domain of the polypeptide comprising SEQ ID NO:2.

77.   The method of claim 68, wherein the agent is a nucleic acid
molecule.

78.   The method of claim 77, wherein the nucleic acid is an
antisense molecule, a ribozyme, a siRNA, or a combination
thereof.

79.   The method of claim 78, wherein the antisense molecule
interacts with a GRP78 polynucleotide.

80.   The method of claim 78, wherein the siRNA interacts with a
GRP78 polynucleotide.

81.   The method of claim 79, wherein the antisense molecule
comprises a sequence that is at least 80% identical to SEQ
ID NO:3 and specifically interacts with a polynucleotide
comprising SEQ ID NO:1.

82.   The method of claim 81, wherein the antisense molecule
comprises a sequence that is at least 90% identical to SEQ
ID NO:3 and specifically interacts with a polynucleotide
comprising SEQ ID NO:1.

83.   The method of claim 81, wherein the antisense molecule
comprises SEQ ID NO:3.

84.   The method of claim 81, wherein the antisense molecule
consists of SEQ ID NO:3.

85.   The method of claim 80, wherein the siRNA comprises a
      sequence that is about 90% identical to SEQ ID NO:4 and its
      complement.

86.   The method of claim 85, wherein the siRNA comprise a
      sequence that is about 95% identical to SEQ ID NO:4 and its
      complement.

87.   The method of claim 85, wherein the siRNA comprises SEQ ID
      NO:4 and its complement.

88.   The method of claim 85, wherein the siRNA consists of SEQ ID
      NO:4 and its complement.

89.   The method of claim 68, wherein the method further comprises
      contacting the cell with a chemotherapeutic agent.

90.   A method of modulating apoptosis, the method comprising
      contacting a cell comprising a glucose regulated protein 94
      (GRP94) endoplasmic reticulum transmembrane protein with an
      agent that regulates the interaction of the transmembrane
      protein with a cytosolic component that mediates apoptosis.

91.   A method of inhibiting apoptosis in a target tissue, the
      method comprising overexpressing GRP78 or GRP94 in said
      tissue.

92.   The method of claim 91, wherein the tissue is neuronal
      tissue.

93.   The method of claim 91, wherein the tissue is vascular
      tissue.

94.   The method of claim 91, wherein the tissue is cardiac
      tissue.

95.   A method of identifying an agent that modulates the
      interaction of glucose regulated protein 94 (GRP94) with a
      cytosolic component that mediates apoptosis, the method
      comprising:

      (a)   providing glucose regulated protein 94 (GRP94);

      (b)   providing a cytosolic component comprising at least
      one caspase;

      (c)   providing an agent;

      (d)   contacting the protein of (a) with the component of
      (b) and the agent of (c) simultaneously or in succession;
      and

(e)   determining the effect of the agent on the interaction of the protein and the component as compared to a control.

96. A nucleic acid construct comprising a glucose regulated protein (GRP) inhibitory nucleic acid molecule operably linked to an expression control element.

97. The nucleic acid construct of claim 96, wherein the nucleic acid is an antisense molecule.

98. The nucleic acid construct of claim 97, wherein the antisense molecule is selected from the group consisting of:

(a)   a nucleic acid comprising a sequence that is at least 80% identical to SEQ ID NO:3 or a fragment and specifically interacts with a GRP polynucleotide;

(b)   a nucleic acid comprising a sequence that is at least 90% identical to SEQ ID NO:3 or a fragment and specifically interacts with a GRP polynucleotide;

(c)   a nucleic acid comprising a sequence that is at least 95% identical to SEQ ID NO:3 or a fragment and specifically interacts with a GRP polynucleotide;

(d)   a nucleic acid comprising a sequence as set forth in SEQ ID NO:3; and

(e)   a nucleic acid consisting of the sequence as set forth in SEQ ID NO:3.

99. The nucleic acid construct of claim 96, wherein the inhibitory nucleic acid molecule is a small inhibitory nucleic acid (siNA) molecule.

100. The nucleic acid construct of claim 99, wherein the siNA molecule is selected from the group consisting of:

(a)   a nucleic acid comprising a sequence that is at least 90% identical to SEQ ID NO:4 and its complement and specifically interacts with a GRP polynucleotide;

(b)   a nucleic acid comprising a sequence that is at least 95% identical to SEQ ID NO:4 and its complement and specifically interacts with a GRP polynucleotide;

(c)   a nucleic acid comprising a sequence as set forth in SEQ ID NO:4 and its complement; and

(d)   a nucleic acid consisting of the sequence set forth in SEQ ID NO:4 and its complement.

101. The nucleic acid construct of claim 96, wherein the
     expression control element comprises a glucose responsive
     protein 78 (grp78) promoter sequence.

102. The nucleic acid construct of claim 101, wherein the grp78
     promoter sequence comprises a sequence from about 3000 base
     pairs 5' of the site of initiation of transcription of the
     grp78 coding sequence to 200 base pairs 3' of the site of
     initiation of the grp78 coding sequence.

103. The nucleic acid construct of claim 99, wherein the siNA
     molecule disrupts expression of an endogenous GRP
     polynucleotide.

104. The nucleic acid construct of claim 103, wherein the
     endogenous GPR polynucleotide is a GRP78 polynucleotide.

105. A recombinant vector comprising the nucleic acid construct
     of claim 96.

106. The recombinant vector of claim 105, wherein the vector is
     an animal cell expression vector.

107. The recombinant vector of claim 105, wherein the vector is a
     viral vector.

108. The recombinant vector of claim 107, wherein the viral
     vector is selected from the group consisting of retroviral
     vectors and DNA viral vectors.

109. A pharmaceutical composition comprising the nucleic acid
     construct of claim 96 in a pharmaceutically acceptable
     carrier.

110. A method for inhibiting cell proliferation comprising
     contacting a target cell having a cell proliferative
     disorder with a nucleic acid construct of claim 96.

111. A method for treating a cell proliferative disorder in a
     subject comprising administering to the subject a nucleic
     acid construct of claim 96.

112. The method of claims 111, wherein the subject is a mammal.

113. The method of claim 112, wherein the mammal is a human.

114. The method of claims 111, wherein the administration is by
     in vivo administration.

115. The method of claim 114, wherein the in vivo administration is by systemic, local, or topical administration.

116. The method of claims 111, wherein the administration is by ex vivo administration.

117. The method of claims 111, wherein the cell proliferative disorder is a neoplastic disorder.

118. The method of claim 117, wherein the neoplastic disorder is selected from the group consisting of lung cancer, colon-rectum cancer, breast cancer, prostate cancer, urinary tract cancer, uterine cancer lymphoma, oral cancer, pancreatic cancer, leukemia, melanoma, stomach cancer, thyroid cancer, liver cancer, and brain cancer and ovarian cancer.

119. A nucleic acid construct comprising a glucose regulated protein (GRP) polynucleotide operably linked to an expression control element.

120. The nucleic acid construct of claim 119, wherein the expression control element comprises a glucose responsive protein 78 (grp78) promoter sequence.

121. The nucleic acid construct of claim 119, wherein the GRP polynucleotide comprises a GRP78 polynucleotide and/or a GRP94 polynucleotide.

122. The nucleic acid construct of claim 121, wherein the GRP polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising a sequence that is at least 80% identical to SEQ ID NO:1, wherein a polypeptide produced from the polynucleotide inhibits apoptosis;

(b) a polynucleotide comprising a sequence that is at least 90% identical to SEQ ID NO:1, wherein a polypeptide produced from the polynucleotide inhibits apoptosis;

(c) a polynucleotide comprising a sequence that is at least 95% identical to SEQ ID NO:1, wherein a polypeptide produced from the polynucleotide inhibits apoptosis; and

(d) a polynucleotide comprising a fragment of SEQ ID NO:1, wherein a polypeptide produced from the polynucleotide inhibits apoptosis.

123. The nucleic acid construct of claim 120, wherein the grp78 promoter sequence comprises a sequence from about 3000 base

pairs 5′ of the site of initiation of transcription of the grp78 coding sequence to 200 base pairs 3′ of the site of initiation of the grp78 coding sequence.

124. A recombinant vector comprising the nucleic acid construct of claim 119.

125. The recombinant vector of claim 124, wherein the vector is an animal cell expression vector.

126. The recombinant vector of claim 124, wherein the vector is a viral vector.

127. The recombinant vector of claim 126, wherein the viral vector is selected from the group consisting of retroviral vectors and DNA viral vectors.

128. A pharmaceutical composition comprising the nucleic acid construct of claim 119 in a pharmaceutically acceptable carrier.

129. A method for inhibiting tissue damage due to ischemia comprising contacting a target cell having a cell susceptible to ischemia with a nucleic acid construct of claim 119.

130. A method for treating ischemic tissue injury in a subject comprising administering to the subject a nucleic acid construct of claim 119.

131. The method of claims 130, wherein the subject is a mammal.

132. The method of claim 131, wherein the mammal is a human.

133. The method of claims 130, wherein the administration is by in vivo administration.

134. The method of claim 133, wherein the in vivo administration is by systemic, local, or topical administration.

135. The method of claims 130, wherein the administration is by ex vivo administration.

Figure 1

Figure 2

Figure 3

A

B
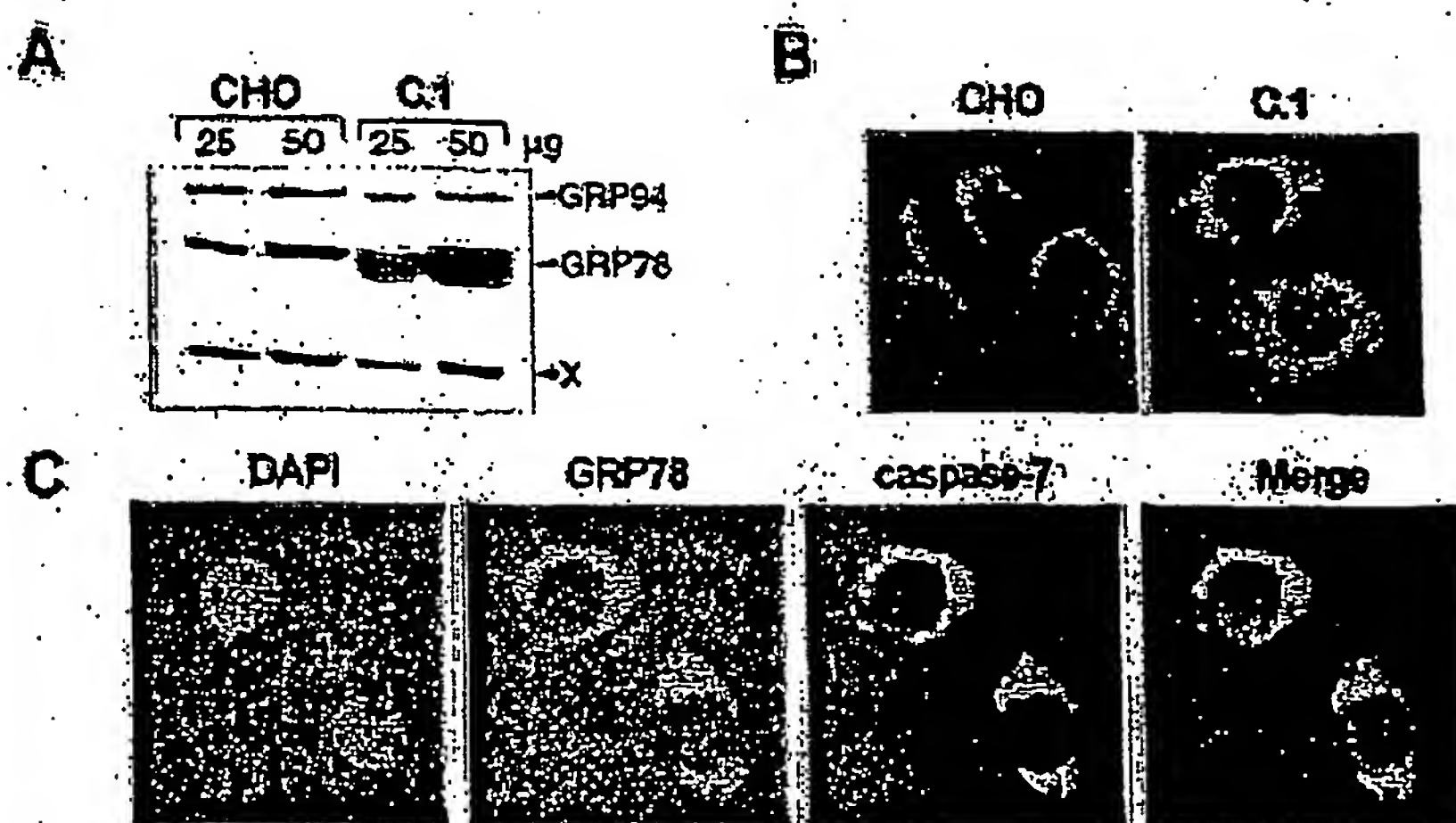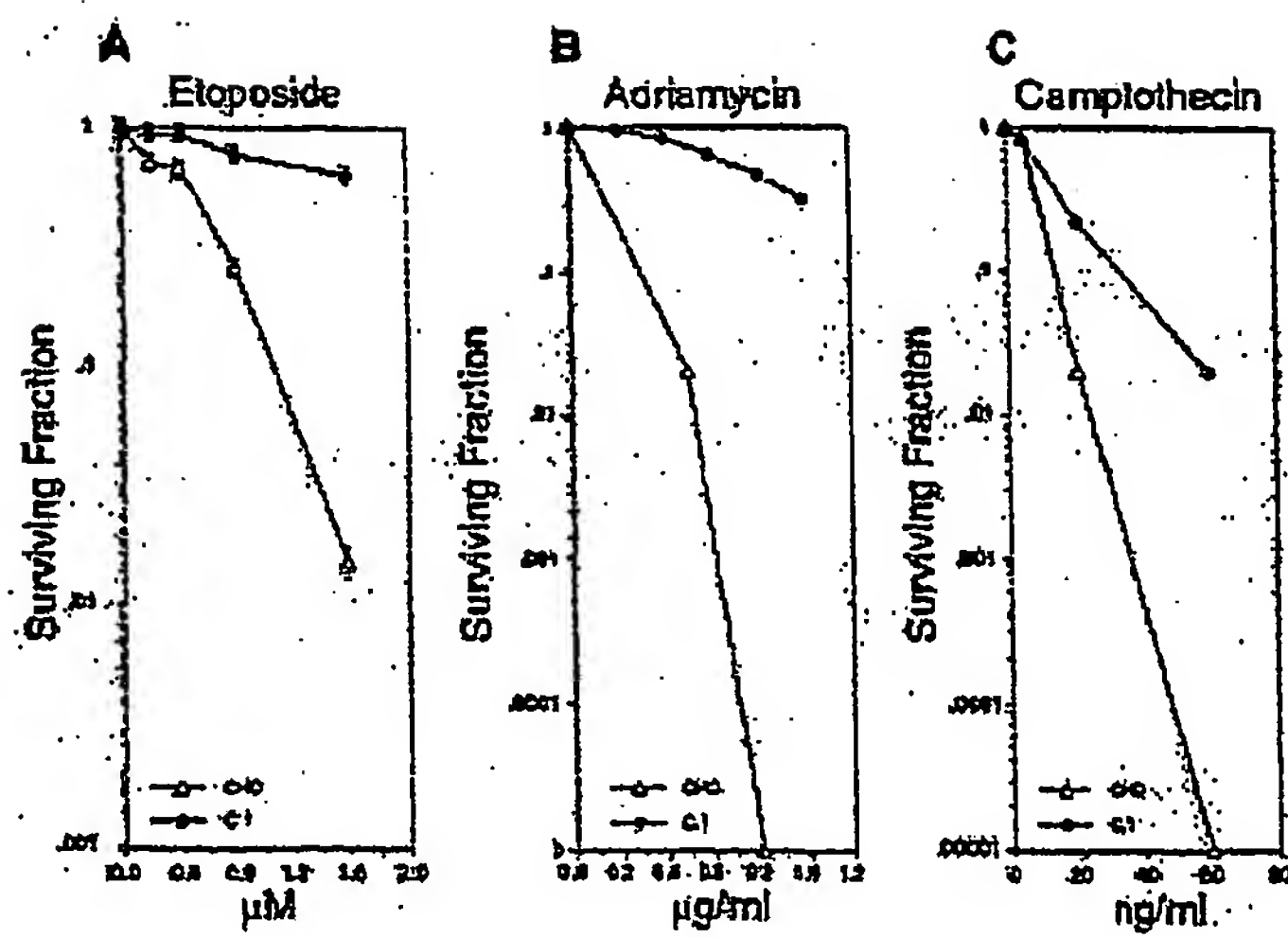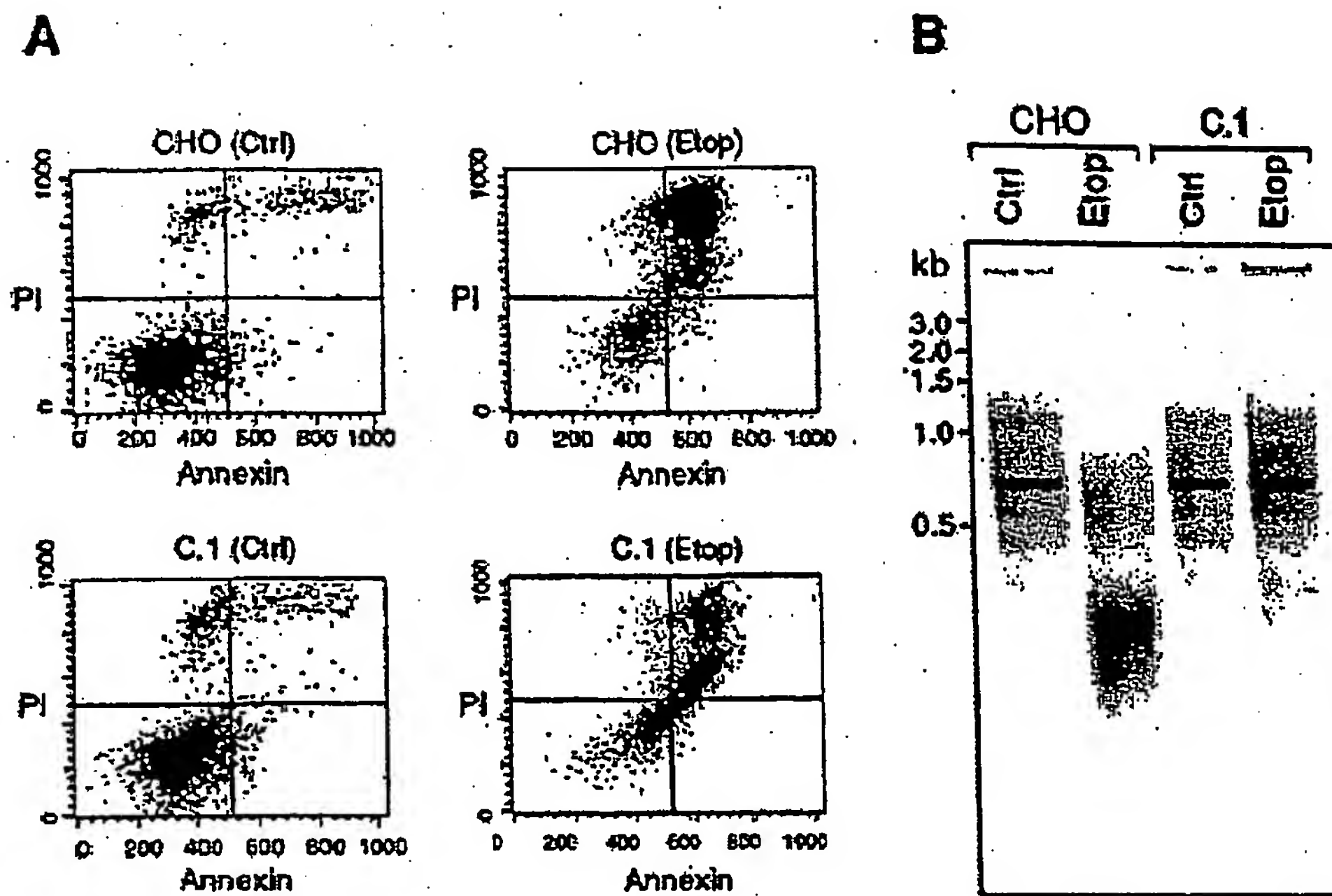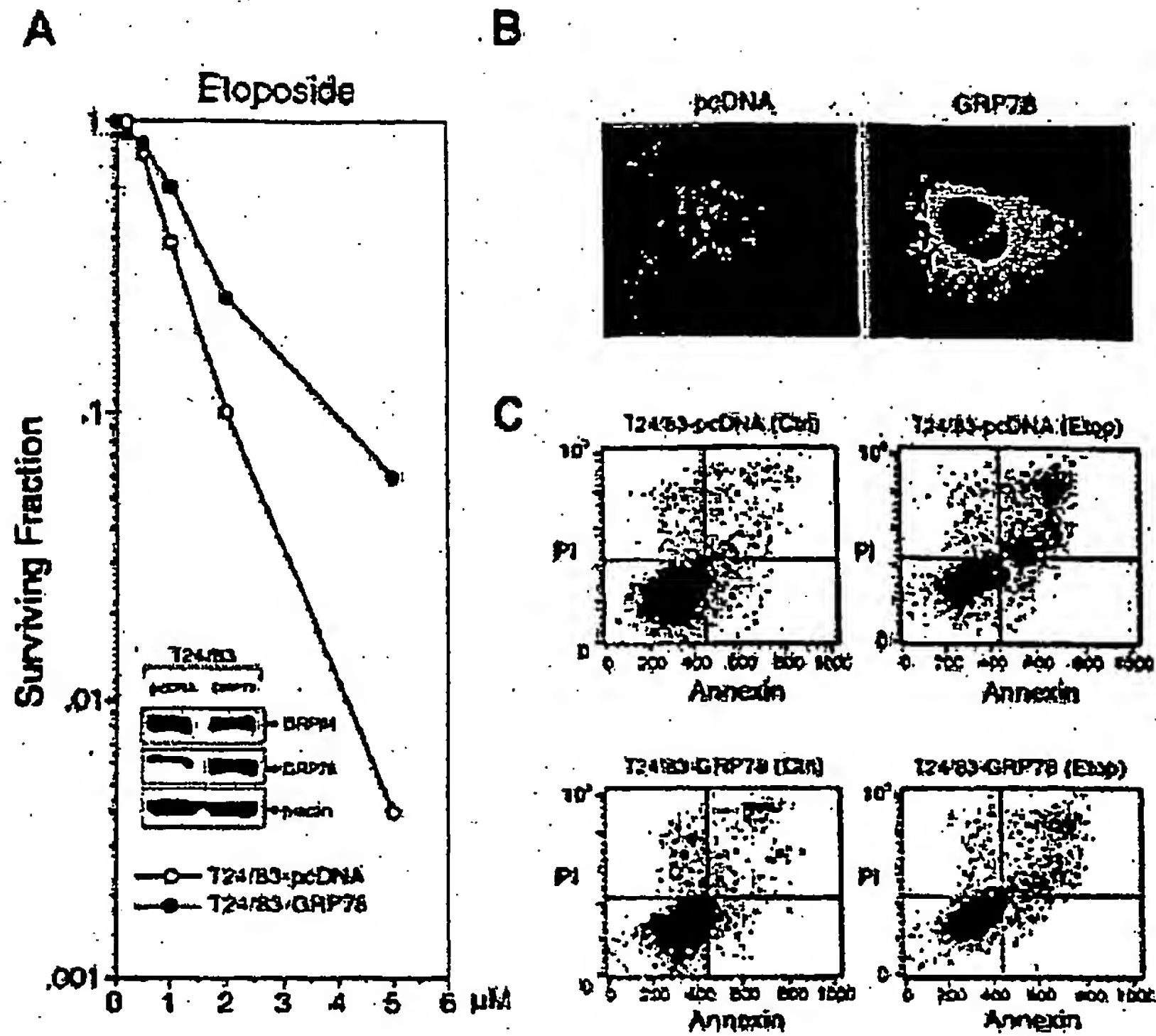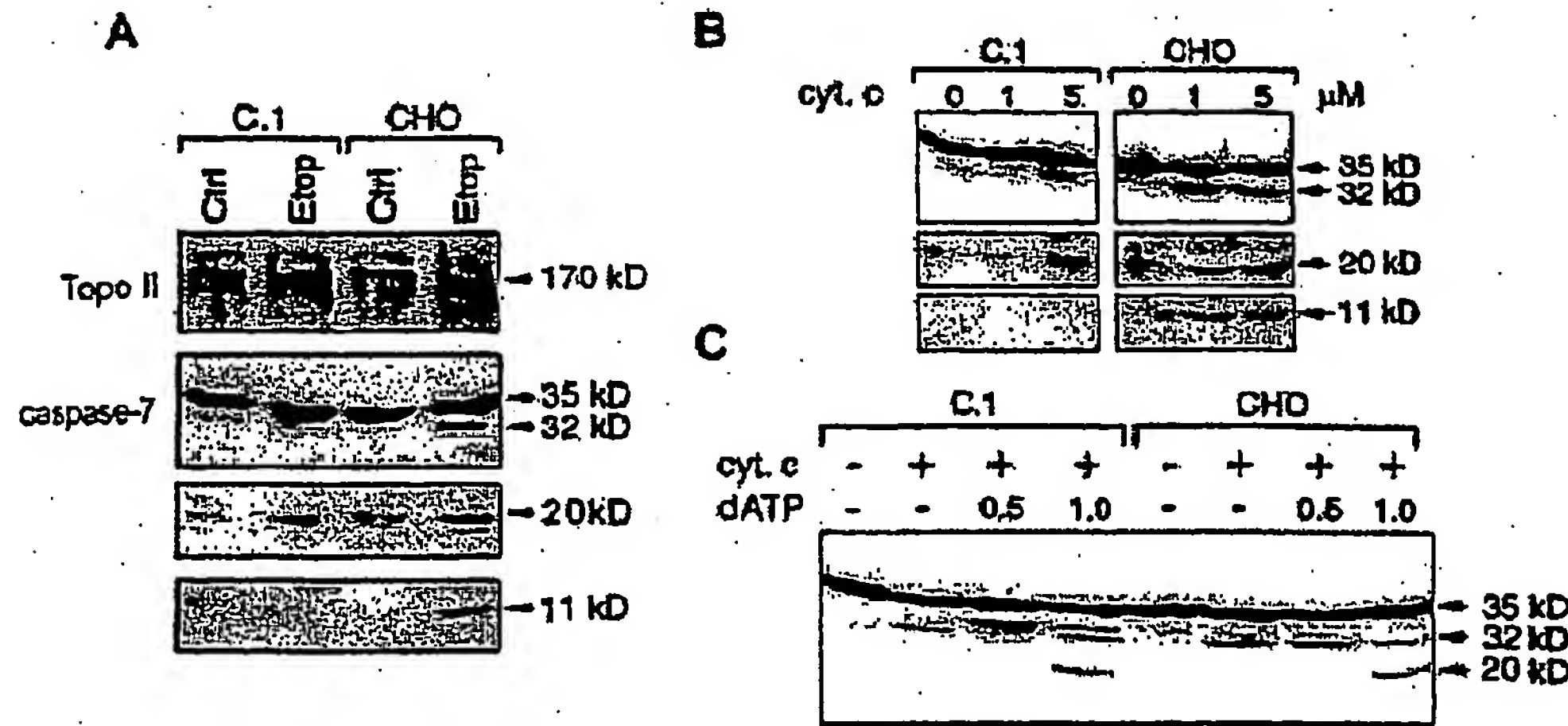
Figure 4

Figure 5

Figure 6
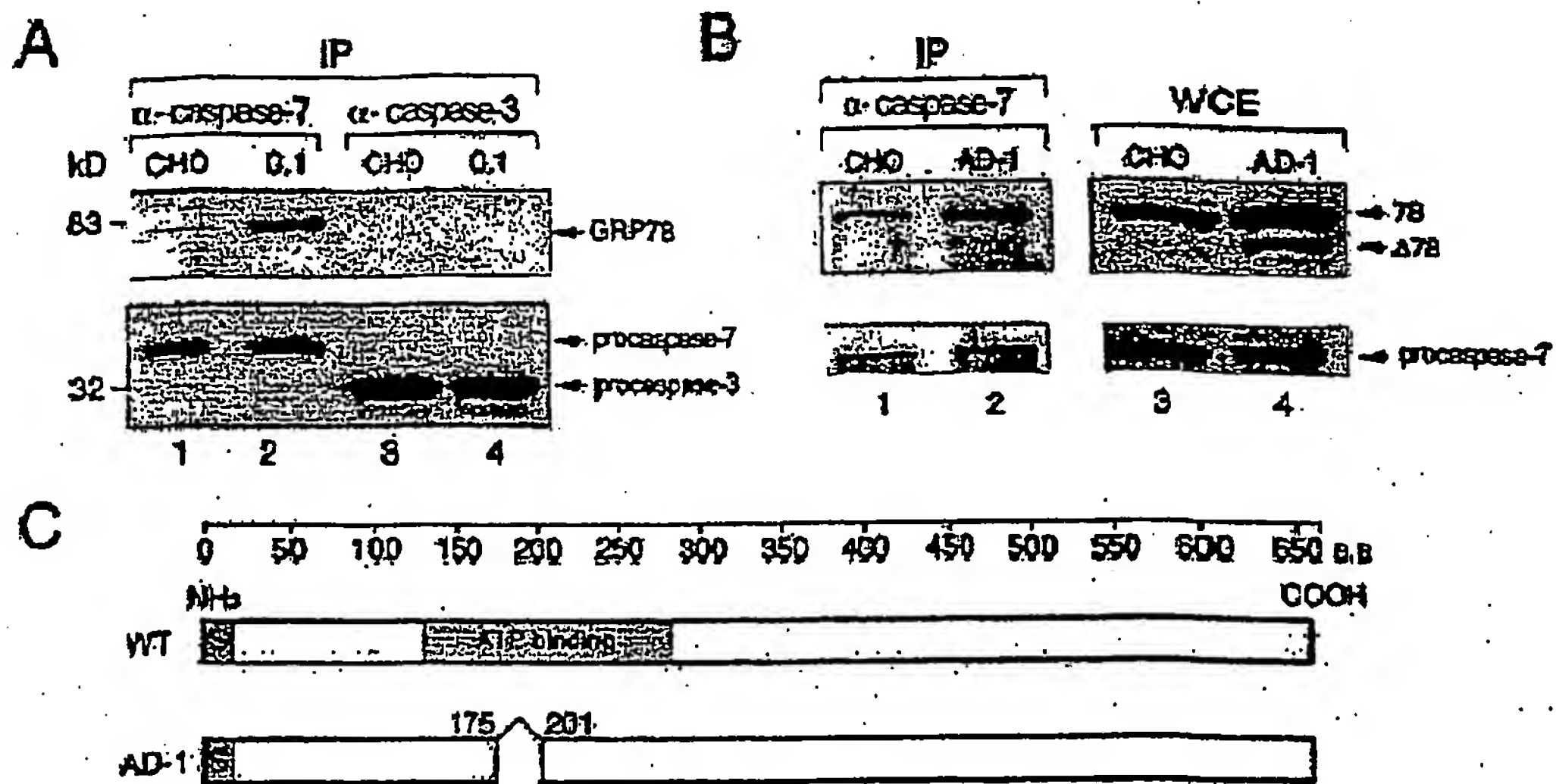
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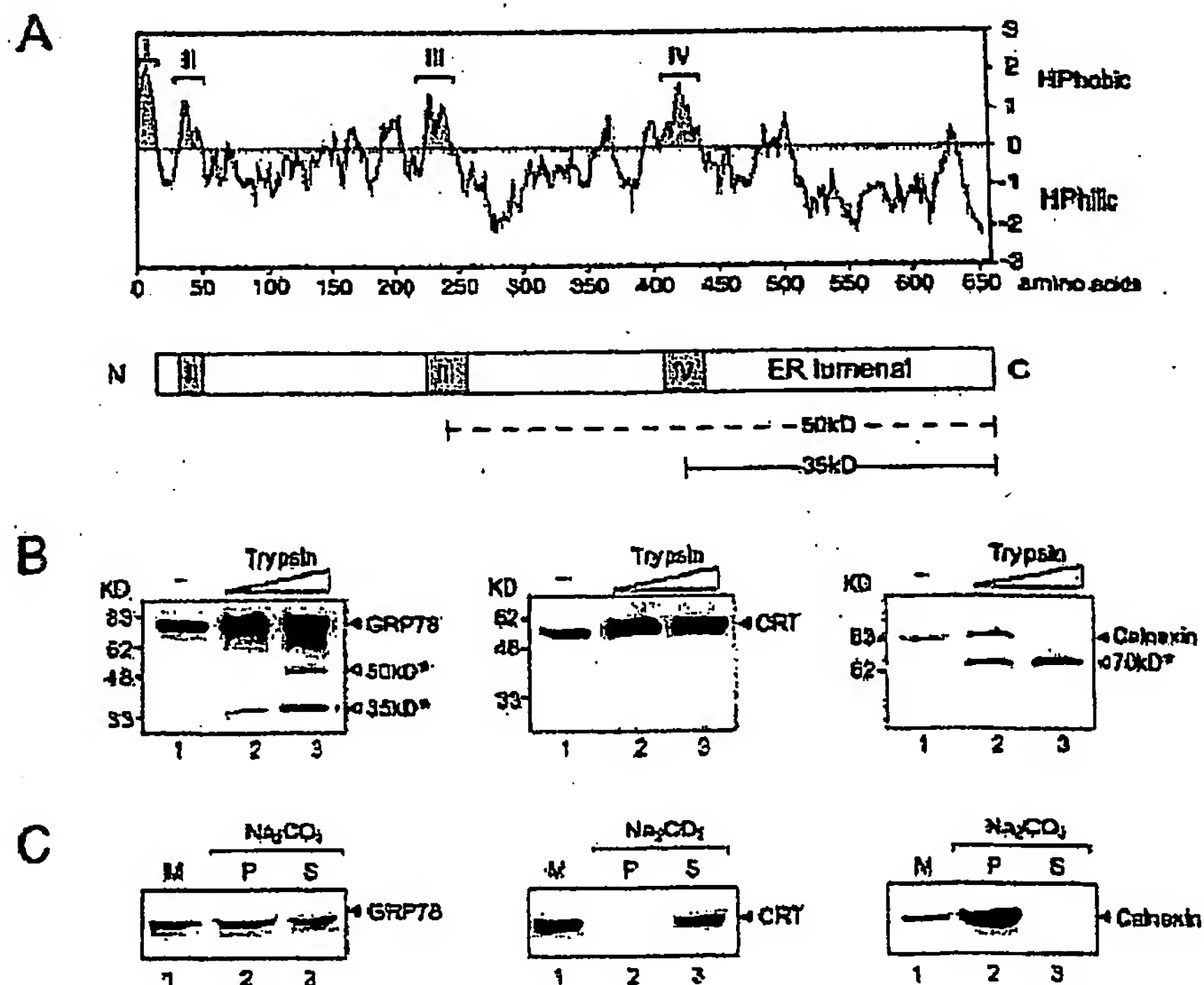
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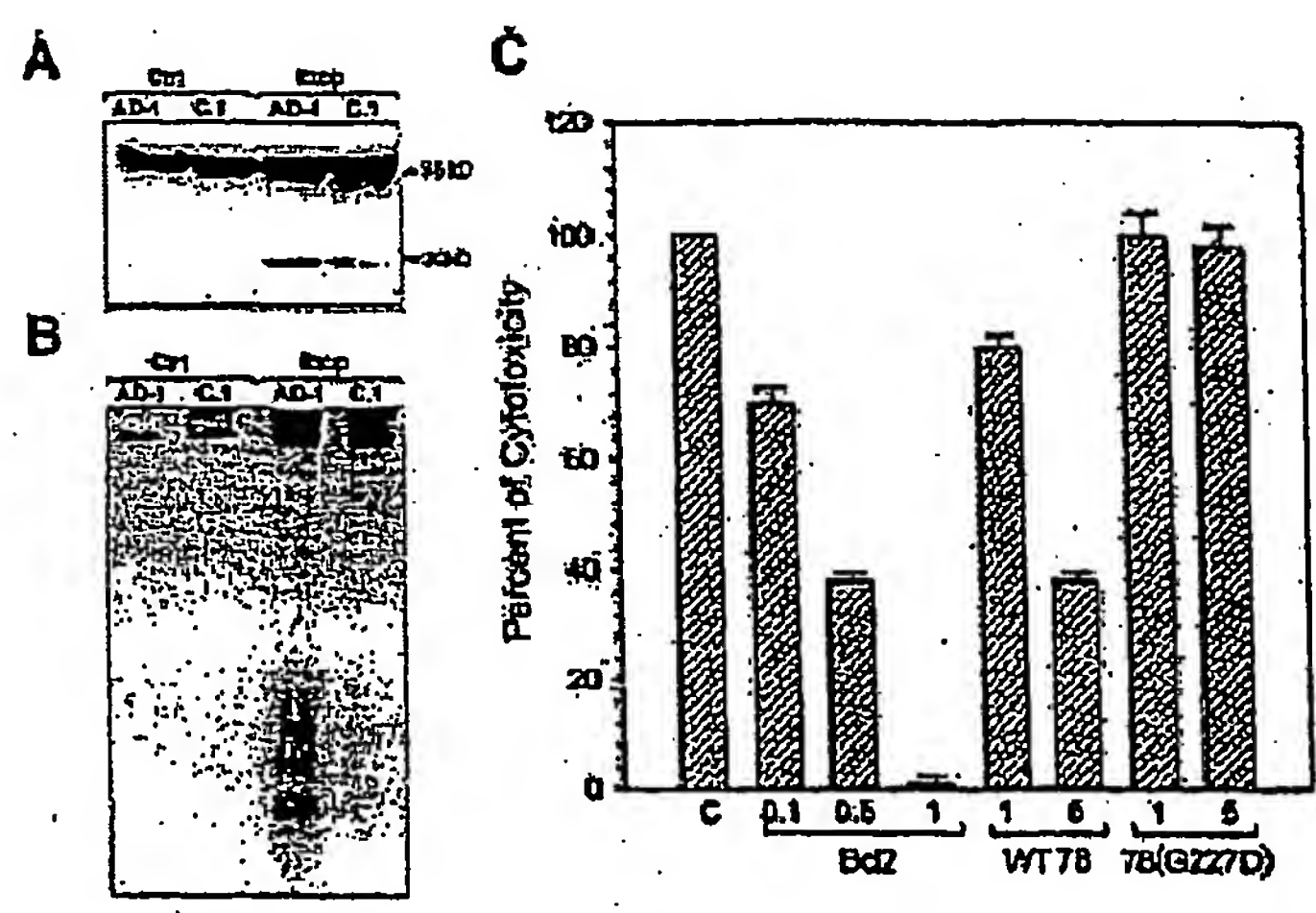
Figure 9

	G_1	S	G_2
CHO	40 ± 5	18 ± 3	30 ± 2
CHO + Tuni	62 ± 8	5 ± 1	24 ± 8
CHO + Tg	55 ± 4	9 ± 2	26 ± 2
C.1	44 ± 4	20 ± 2	28 ± 6
T24/83-pcDNA	52 ± 7	20 ± 8	30 ± 6
T24/83-pcDNA + Tuni	68 ± 8	8 ± 2	28 ± 2
T24/83-pcDNA + Tg	77 ± 4	6 ± 2	16 ± 2
T24/83-GRP78	50 ± 4	21 ± 9	28 ± 4
T24/83-GRP78 + Tuni	76 ± 8	6 ± 2	22 ± 8
T24/83-GRP78 + Tg	72 ± 4	9 ± 3	22 ± 2

Figure 10

Phu 6.5
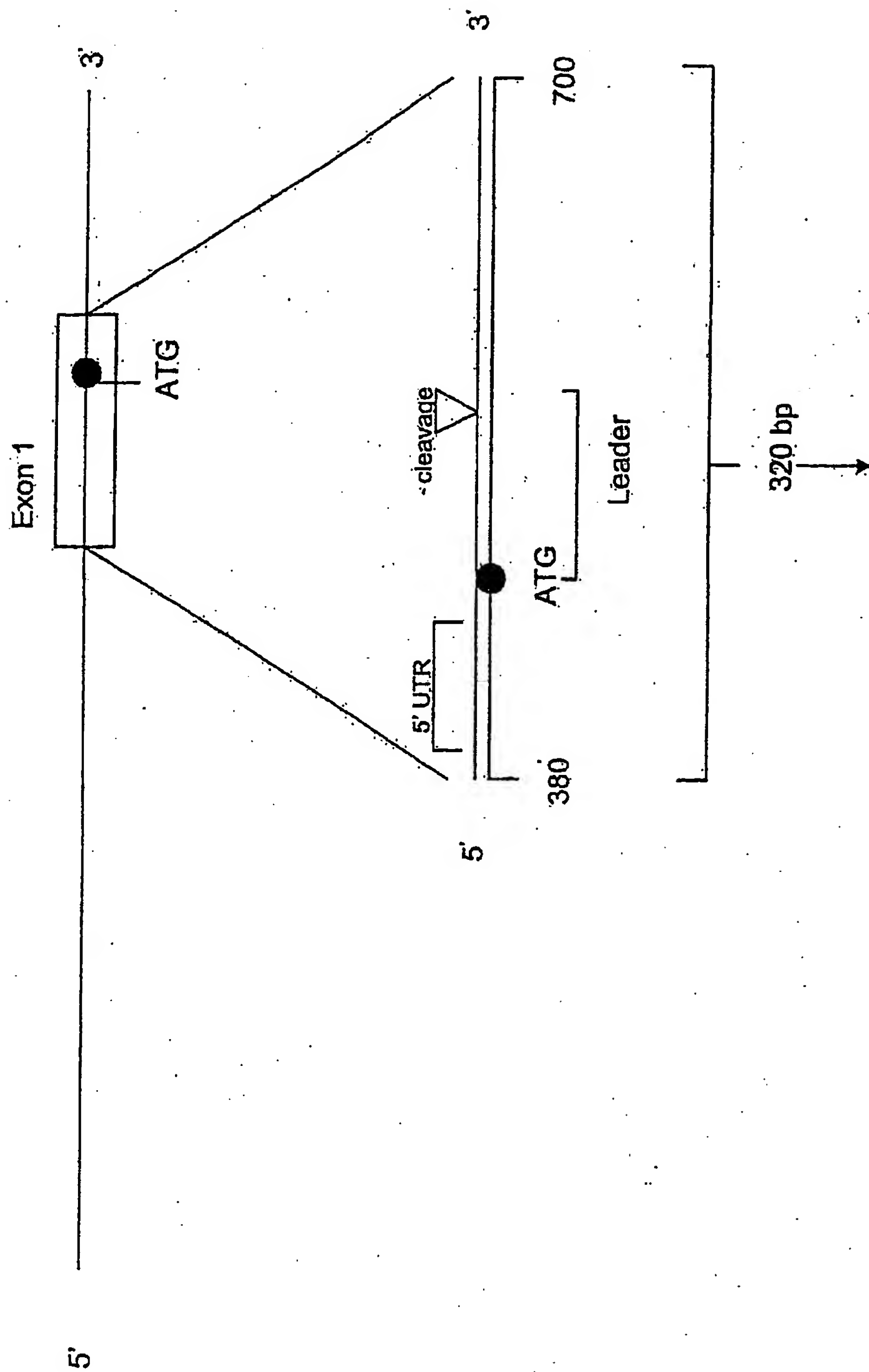
Clone into adenovirus vector in reverse orientation

Figure 2. Scheme for subcloning the 320 bp grp78 exon I fragment.
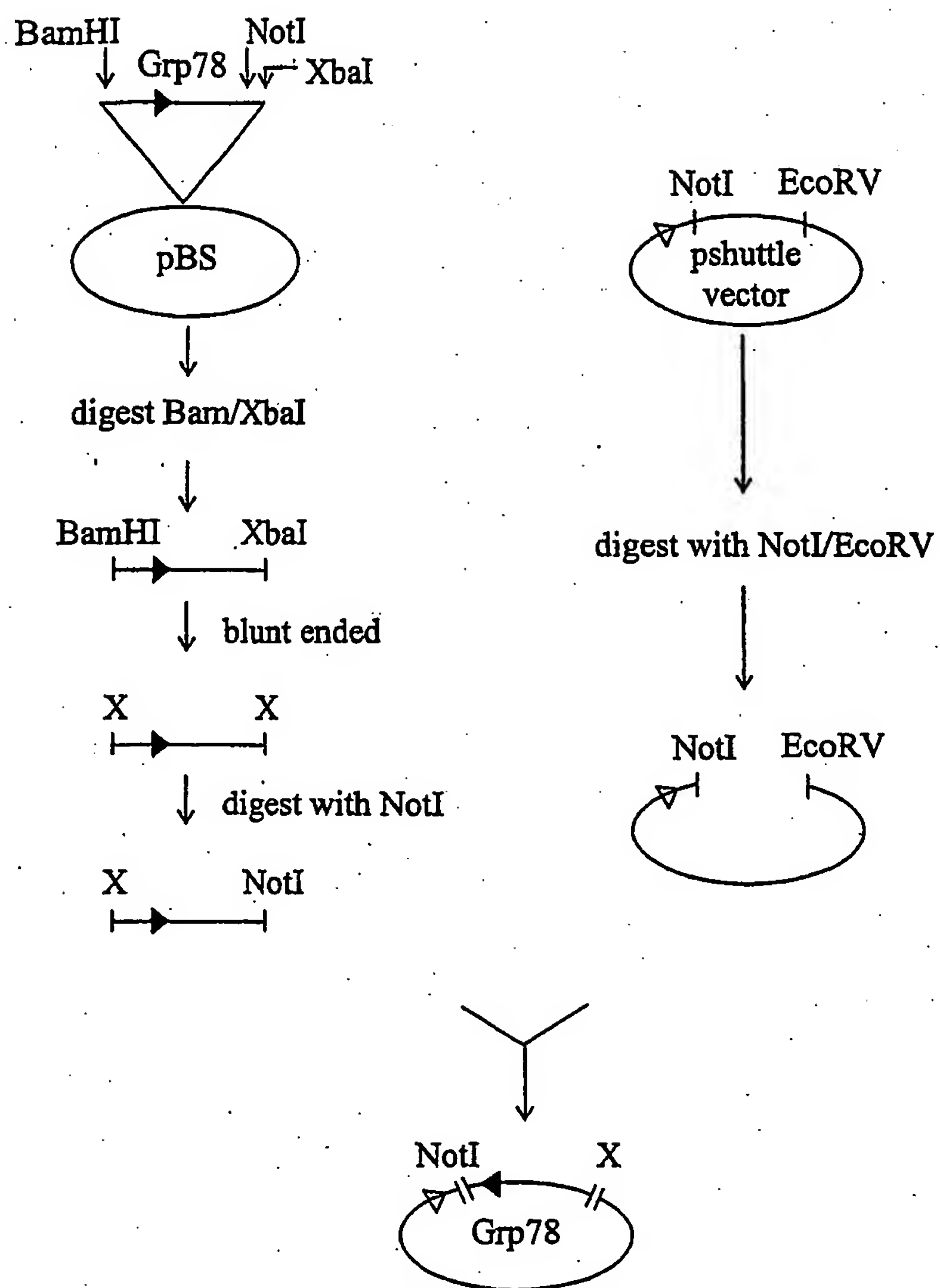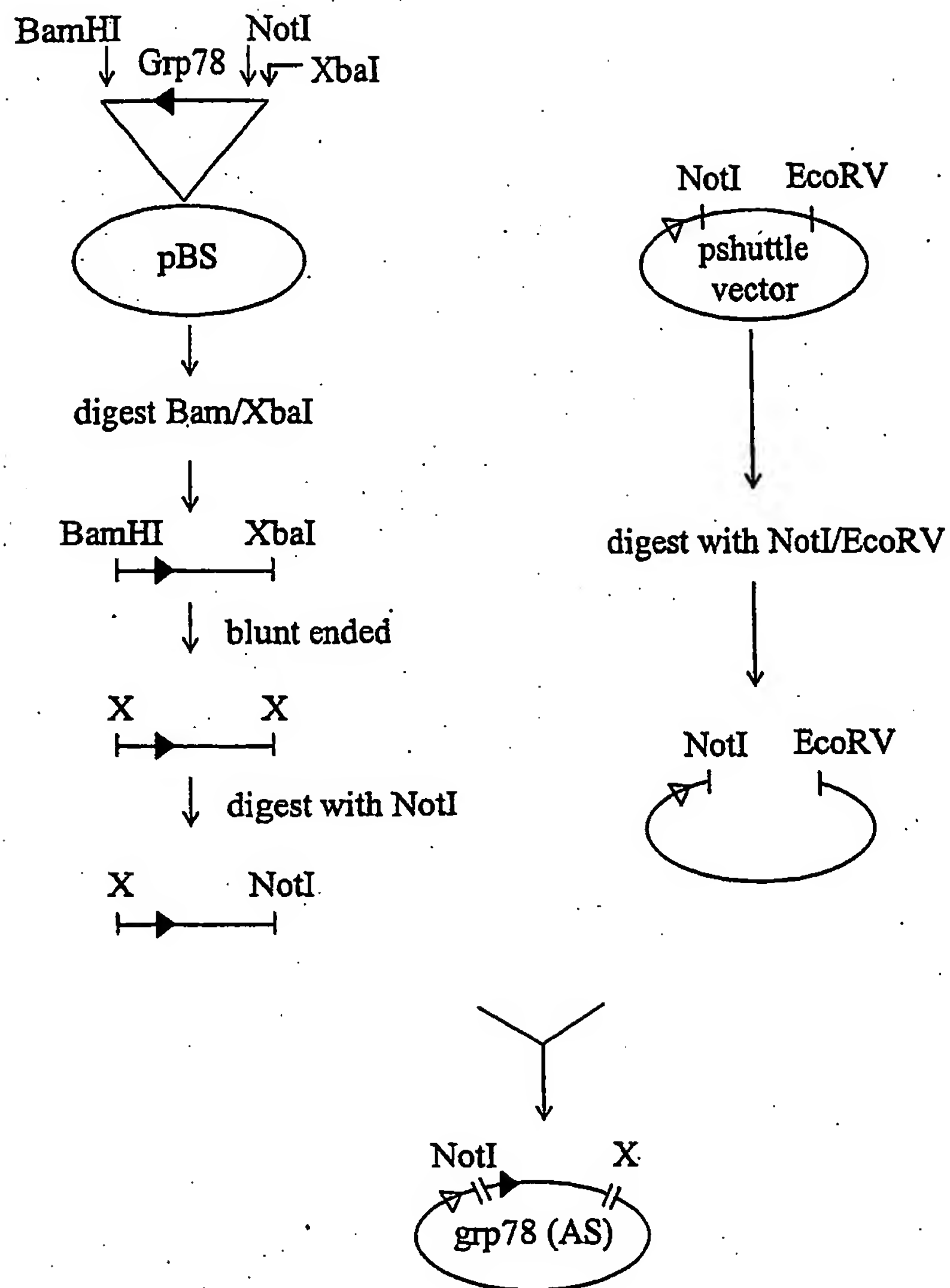
FIG. 11A

FIG. 11B
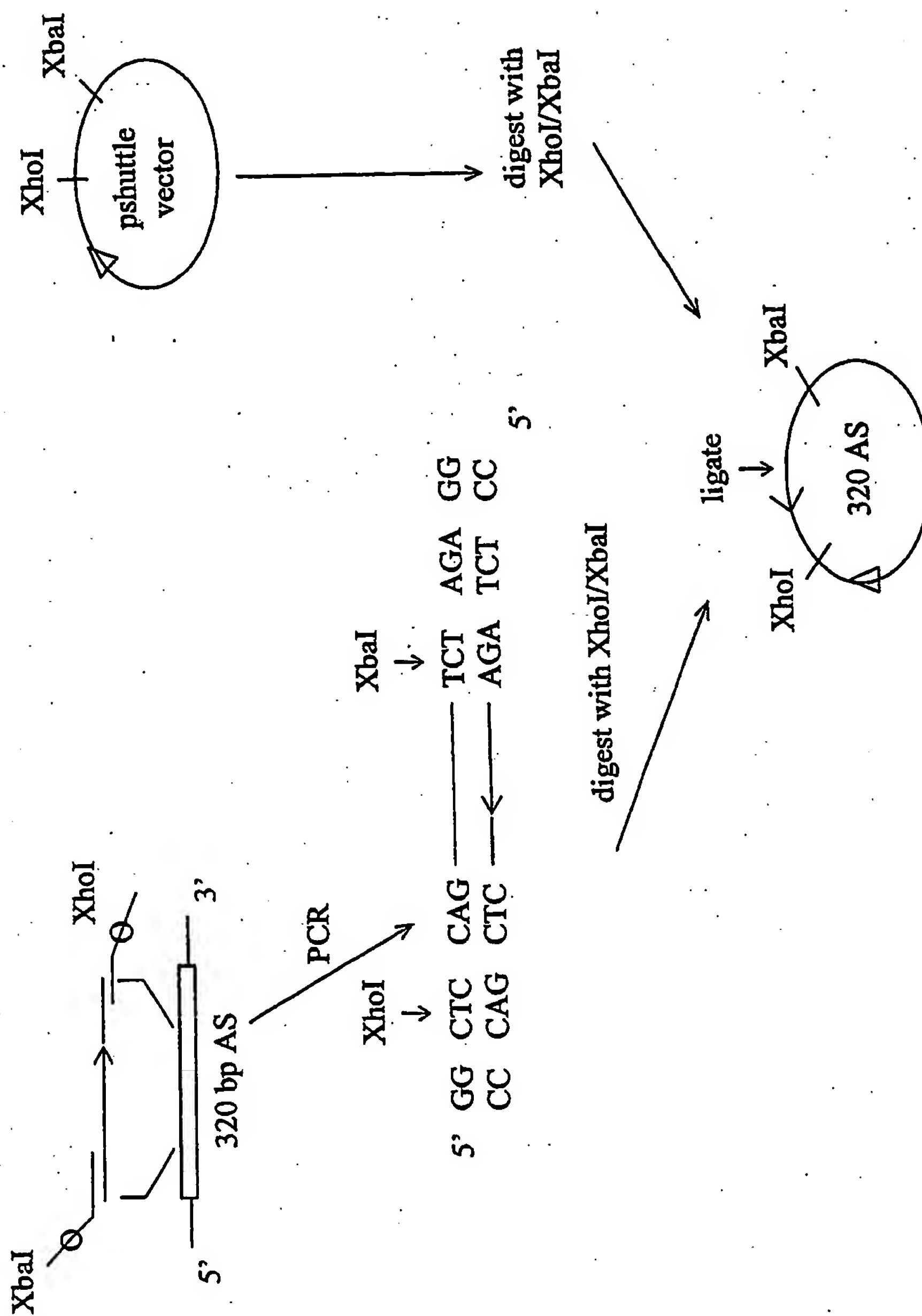
FIG. 11C
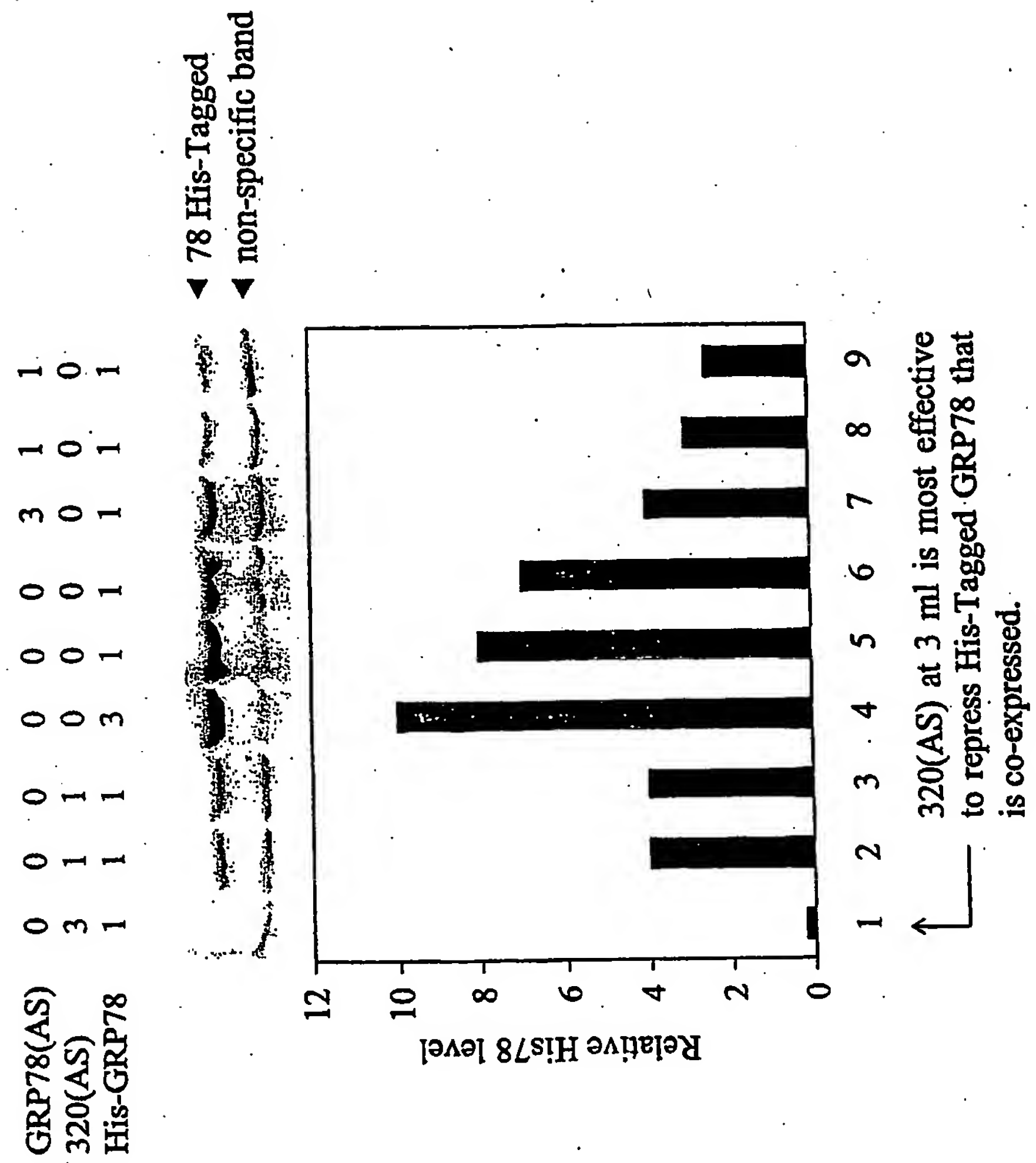
FIG. 12
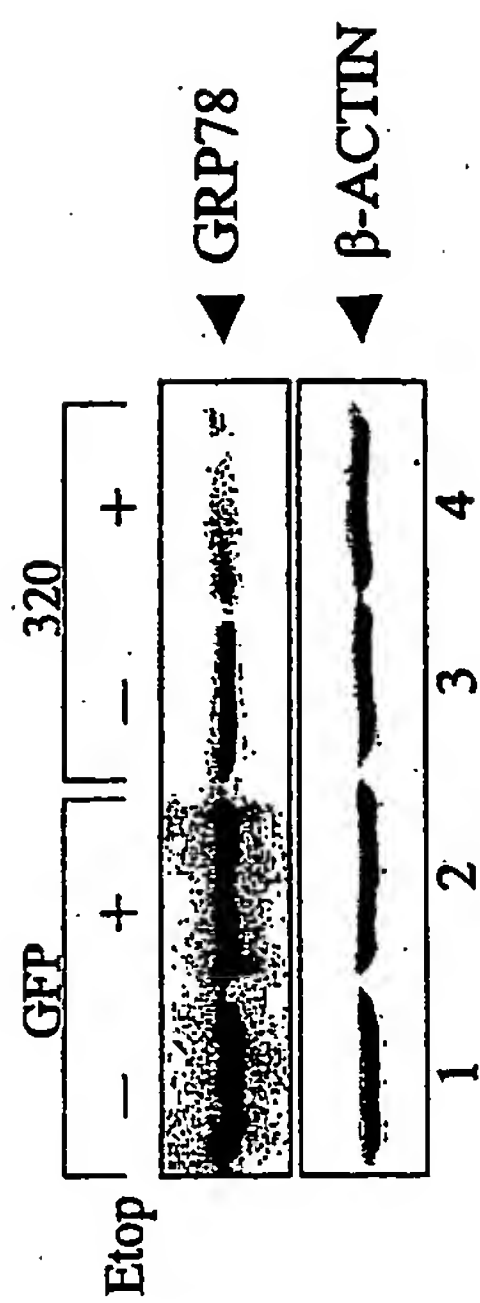
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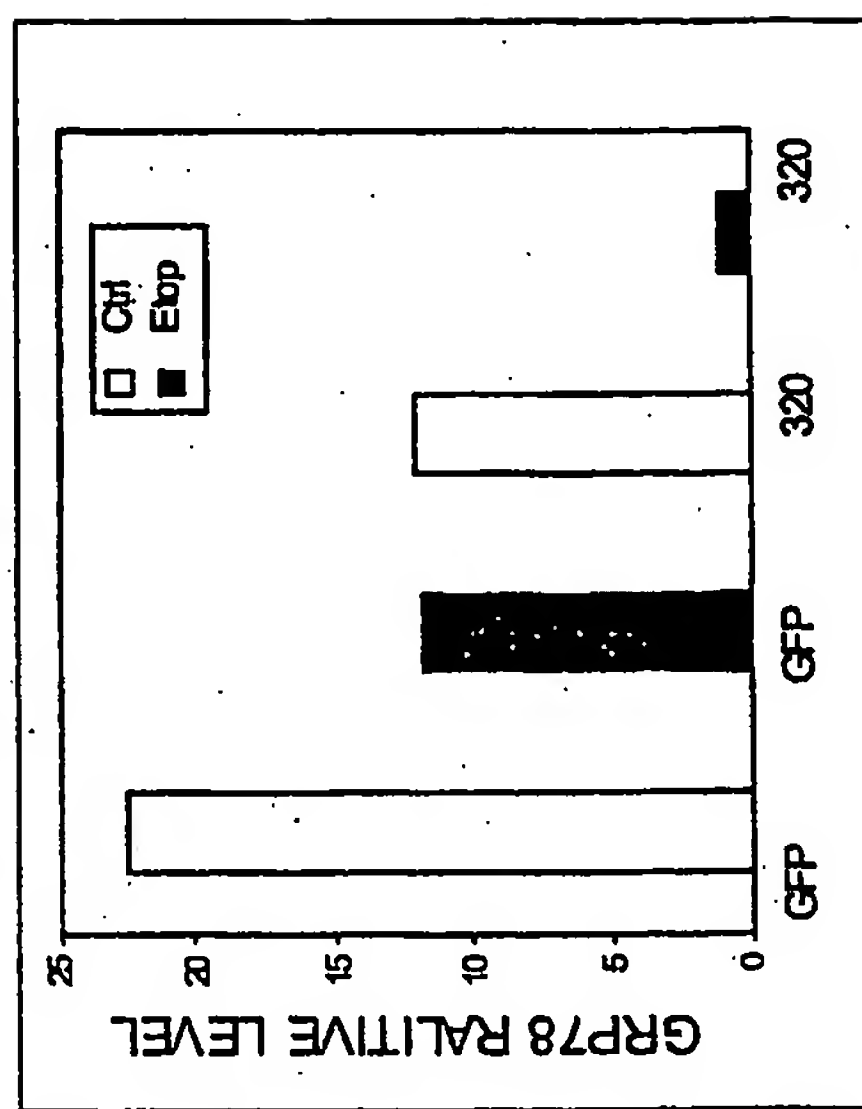
FIG. 13B

TRANSDUCED MDA-MB-435 CELLS: Adenovirus supernatant 0.5 ml/well (6-well dish), 68 hrs
ETOPOSIDE TREATMENT: 20 μm, 6 hrs, recovered for 4 hrs

FIG. 14

FIG. 15
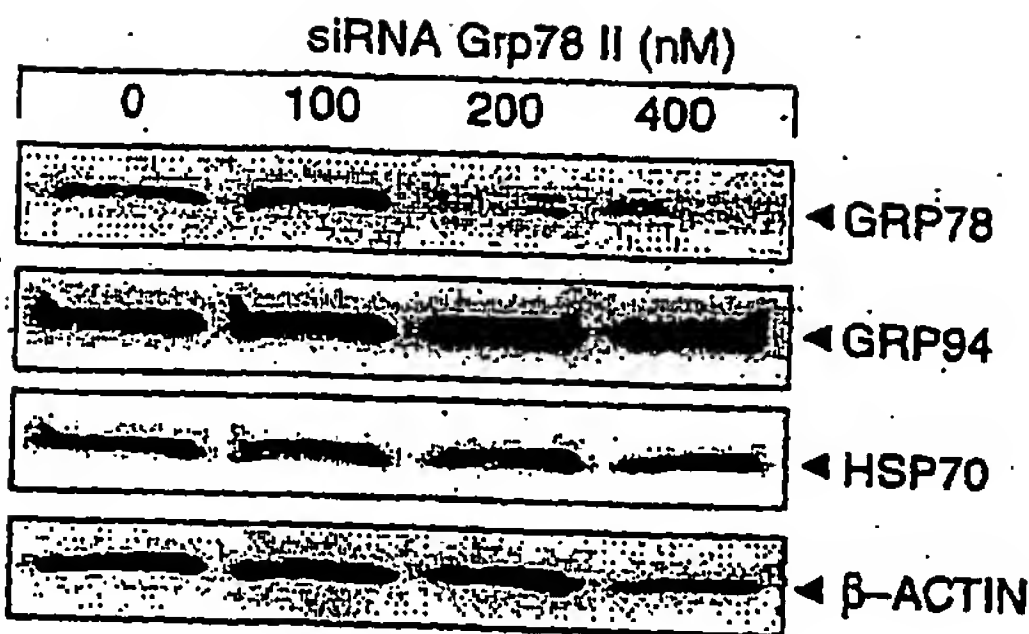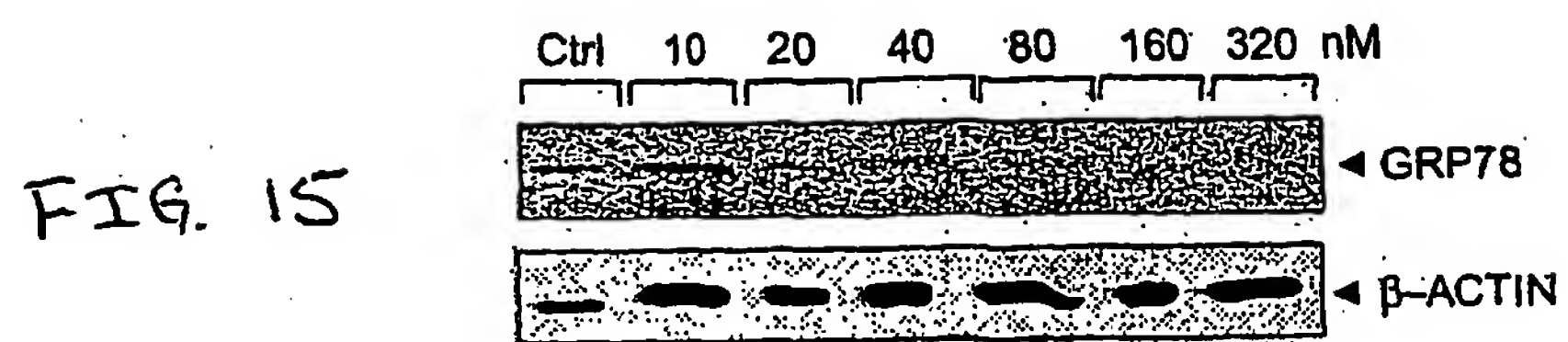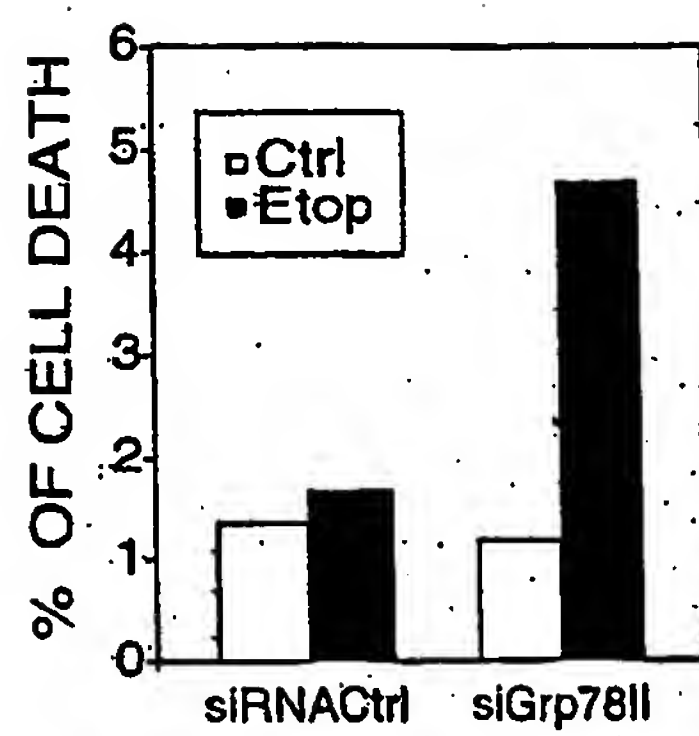
FIG. 16
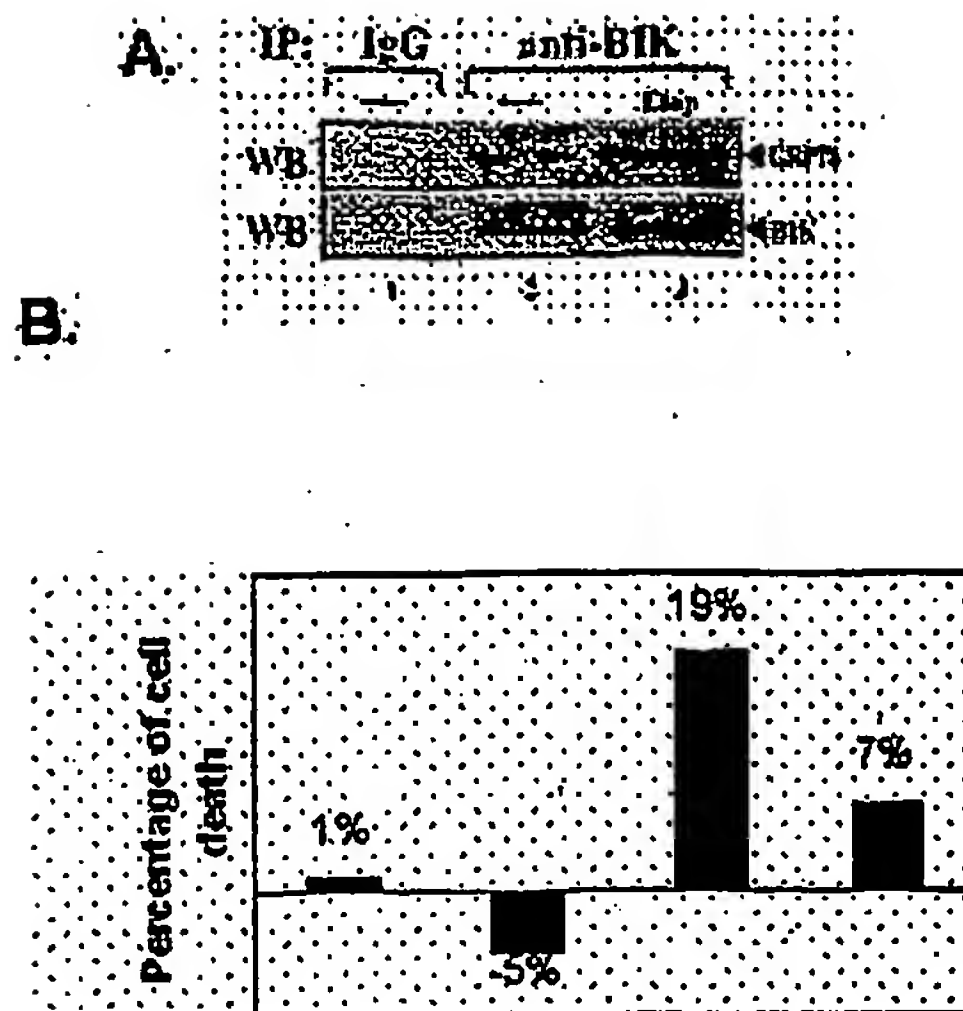
Fig. 17 A-B

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ggc aac cgc atc acg ccg tcc tat gtc gcc ttc act cct gaa ggg gaa   423
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         60   65   70

cgt ctg att ggc gat gcc gcc aag aac cag ctc acc tcc aac ccc gag   471
Arg Leu Ile Gly Asp Ala Ala Lys Asn Gln Leu Thr Ser Asn Pro Glu
     75   80   85

aac acg gtc ttt gac gcc aag cgg ctc atc ggc cgc acg tgg aat gac   519
Asn Thr Val Phe Asp Ala Lys Arg Leu Ile Gly Arg Thr Trp Asn Asp
 90   95   100   105

ccg tct gtg cag cag gac atc aag ttc ttg ccg ttc aag gtg gtt gaa   567
Pro Ser Val Gln Gln Asp Ile Lys Phe Leu Pro Phe Lys Val Val Glu
                 110   115   120

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aaa gaa acc gct gag gct tat ttg gga aag aag gtt acc cat gca gtt    711
Lys Glu Thr Ala Glu Ala Tyr Leu Gly Lys Lys Val Thr His Ala Val
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gtt act gta cca gcc tat ttt aat gat gcc caa cgc caa gca acc aaa    759
Val Thr Val Pro Ala Tyr Phe Asn Asp Ala Gln Arg Gln Ala Thr Lys
170             175             180             185

gac gct gga act att gct ggc cta aat gtt atg agg atc atc aac gag    807
Asp Ala Gly Thr Ile Ala Gly Leu Asn Val Met Arg Ile Ile Asn Glu
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cct acg gca gct gct att gct tat ggc ctg gat aag agg gag ggg gag    855
Pro Thr Ala Ala Ala Ile Ala Tyr Gly Leu Asp Lys Arg Glu Gly Glu
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Phe Ile Lys Leu Tyr Lys Lys Lys Thr Gly Lys Asp Val Arg Lys Asp
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Phe Phe Asn Gly Lys Glu Pro Ser Arg Gly Ile Asn Pro Asp Glu Ala
        380             385             390

gta gcg tat ggt gct gct gtc cag gct ggt gtg ctc tct ggt gat caa       1431
Val Ala Tyr Gly Ala Ala Val Gln Ala Gly Val Leu Ser Gly Asp Gln
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Lys Asp Asn His Leu Leu Gly Thr Phe Asp Leu Thr Gly Ile Pro Pro
        475             480             485

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Tyr Val Ala Phe Thr Pro Glu Gly Glu Arg Leu Ile Gly Asp Ala Ala
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Lys Phe Leu Pro Phe Lys Val Val Glu Lys Lys Thr Lys Pro Tyr Ile
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Gln Val Asp Ile Gly Gly Gly Gln Thr Lys Thr Phe Ala Pro Glu Glu
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gaaggtgaac acacacccta acccaggttt tttacctgct ttttaagatg gccaattctt    1560
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gagggcctgc acttccatag agtttgctga taattggttg aacaatttct tccagttcct    1860
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gacatacatc aagcagtacc aggtcacctg tatcttgatc accagagagc acaccagcct    2520
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gcttcatagt agaccggaac agatccatgt tgagctcttc aaatttggcc cgagtcaggg    2760
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agcgcaattt ccgacttgca ggcggcaggg gcccggggtc acaaggcgcc acgaaccagg    3840
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<213> Homo sapiens

<400> 4
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<212> DNA
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tccggccgtc cccaccccac ctcgccgcc atg cgc ctc cgc cgc cta gcg ctg     113
                                 Met Arg Leu Arg Arg Leu Ala Leu
                                 1               5


ttc ccg ggt gtg gcg ctg ctt ctt gcc gcg gcc cgc ctc gcc gct gcc    161
Phe Pro Gly Val Ala Leu Leu Leu Ala Ala Ala Arg Leu Ala Ala Ala
    10              15                  20


tcc gac gtg cta gaa ctc acg gac gac aac ttc gag agt cgc atc tcc    209
Ser Asp Val Leu Glu Leu Thr Asp Asp Asn Phe Glu Ser Arg Ile Ser
25                  30                  35                  40


gac acg ggc tct gcg ggc ctc atg ctc gtc gag ttc ttc gcc ccc tgg    257
Asp Thr Gly Ser Ala Gly Leu Met Leu Val Glu Phe Phe Ala Pro Trp
                    45                  50                  55


tgt gga cac tgc aag aga ctt gca cct gag tat gaa gct gca gct acc    305
Cys Gly His Cys Lys Arg Leu Ala Pro Glu Tyr Glu Ala Ala Ala Thr
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```
                    60                    65                    70

aga tta aaa gga ata gtc cca tta gca aag gtt gat tgc act gcc aac        353
Arg Leu Lys Gly Ile Val Pro Leu Ala Lys Val Asp Cys Thr Ala Asn
            75                  80                  85

act aac acc tgt aat aaa tat gga gtc agt gga tat cca acc ctg aag        401
Thr Asn Thr Cys Asn Lys Tyr Gly Val Ser Gly Tyr Pro Thr Leu Lys
            90                  95                  100

ata ttt aga gat ggt gaa gaa gca ggt gct tat gat gga cct agg act        449
Ile Phe Arg Asp Gly Glu Glu Ala Gly Ala Tyr Asp Gly Pro Arg Thr
105                 110                 115                 120

gct gat gga att gtc agc cac ttg aag aag cag gca gga cca gct tca        497
Ala Asp Gly Ile Val Ser His Leu Lys Lys Gln Ala Gly Pro Ala Ser
            125                 130                 135

gtg cct ctc agg act gag gaa gaa ttt aag aaa ttc att agt gat aaa        545
Val Pro Leu Arg Thr Glu Glu Glu Phe Lys Lys Phe Ile Ser Asp Lys
            140                 145                 150

gat gcc tct ata gta ggt ttt ttc gat gat tca ttc agt gag gct cac        593
Asp Ala Ser Ile Val Gly Phe Phe Asp Asp Ser Phe Ser Glu Ala His
            155                 160                 165

tcc gag ttc cta aaa gca gcc agc aac ttg agg gat aac tac cga ttt        641
Ser Glu Phe Leu Lys Ala Ala Ser Asn Leu Arg Asp Asn Tyr Arg Phe
            170                 175                 180

gca cat acg aat gtt gag tct ctg gtg aac gag tat gat gat aat gga        689
Ala His Thr Asn Val Glu Ser Leu Val Asn Glu Tyr Asp Asp Asn Gly
185                 190                 195                 200

gag ggt atc atc tta ttt cgt cct tca cat ctc act aac aag ttt gag        737
Glu Gly Ile Ile Leu Phe Arg Pro Ser His Leu Thr Asn Lys Phe Glu
            205                 210                 215

gac aag act gtg gca tat aca gag caa aaa atg acc agt ggc aaa att        785
Asp Lys Thr Val Ala Tyr Thr Glu Gln Lys Met Thr Ser Gly Lys Ile
            220                 225                 230

aaa aag ttt atc cag gaa aac att ttt ggt atc tgc cct cac atg aca        833
Lys Lys Phe Ile Gln Glu Asn Ile Phe Gly Ile Cys Pro His Met Thr
            235                 240                 245

gaa gac aat aaa gat ttg ata cag ggc aag gac tta ctt att gct tac        881
Glu Asp Asn Lys Asp Leu Ile Gln Gly Lys Asp Leu Leu Ile Ala Tyr
            250                 255                 260

tat gat gtg gac tat gaa aag aac gct aaa ggt tcc aac tac tgg aga        929
Tyr Asp Val Asp Tyr Glu Lys Asn Ala Lys Gly Ser Asn Tyr Trp Arg
265                 270                 275                 280

aac agg gta atg atg gtg gca aag aaa ttc ctg gat gct ggg cac aaa        977
Asn Arg Val Met Met Val Ala Lys Lys Phe Leu Asp Ala Gly His Lys
            285                 290                 295

ctc aac ttt gct gta gct agc cgc aaa acc ttt agc cat gaa ctt tct       1025
Leu Asn Phe Ala Val Ala Ser Arg Lys Thr Phe Ser His Glu Leu Ser
            300                 305                 310
```

```
gat ttt ggc ttg gag agc act gct gga gag att cct gtt gtt gct atc      1073
Asp Phe Gly Leu Glu Ser Thr Ala Gly Glu Ile Pro Val Val Ala Ile
        315             320             325

aga act gct aaa gga gag aag ttt gtc atg cag gag gag ttc tcg cgt      1121
Arg Thr Ala Lys Gly Glu Lys Phe Val Met Gln Glu Glu Phe Ser Arg
        330             335             340

gat ggg aag gct ctg gag agg ttc ctg cag gat tac ttt gat ggc aat      1169
Asp Gly Lys Ala Leu Glu Arg Phe Leu Gln Asp Tyr Phe Asp Gly Asn
345             350             355             360

ctg aag aga tac ctg aag tct gaa cct atc cca gag agc aat gat ggg      1217
Leu Lys Arg Tyr Leu Lys Ser Glu Pro Ile Pro Glu Ser Asn Asp Gly
                365             370             375

cct gtg aag gta gtg gta gca gag aat ttt gat gaa ata gtg aat aat      1265
Pro Val Lys Val Val Val Ala Glu Asn Phe Asp Glu Ile Val Asn Asn
                380             385             390

gaa aat aaa gat gtg ctg att gaa ttt tat gcc cct tgg tgt ggt cac      1313
Glu Asn Lys Asp Val Leu Ile Glu Phe Tyr Ala Pro Trp Cys Gly His
        395             400             405

tgt aag aac ctg gag ccc aag tat aaa gaa ctt ggc gag aag ctc agc      1361
Cys Lys Asn Leu Glu Pro Lys Tyr Lys Glu Leu Gly Glu Lys Leu Ser
        410             415             420

aaa gac cca aat atc gtc ata gcc aag atg gat gcc aca gcc aat gat      1409
Lys Asp Pro Asn Ile Val Ile Ala Lys Met Asp Ala Thr Ala Asn Asp
425             430             435             440

gtg cct tct cca tat gaa gtc aga ggt ttt cct acc ata tac ttc tct      1457
Val Pro Ser Pro Tyr Glu Val Arg Gly Phe Pro Thr Ile Tyr Phe Ser
            445             450             455

cca gcc aac aag aag cta aat cca aag aaa tat gaa ggt ggc cgt gaa      1505
Pro Ala Asn Lys Lys Leu Asn Pro Lys Lys Tyr Glu Gly Gly Arg Glu
            460             465             470

tta agt gat ttt att agc tat cta caa aga gaa gct aca aac ccc cct      1553
Leu Ser Asp Phe Ile Ser Tyr Leu Gln Arg Glu Ala Thr Asn Pro Pro
            475             480             485

gta att caa gaa gaa aaa ccc aag aag aag aag aag gca cag gag gat      1601
Val Ile Gln Glu Glu Lys Pro Lys Lys Lys Lys Lys Ala Gln Glu Asp
        490             495             500

ctc taaagcagta gccaaacacc actttgtaaa aggactcttc catcagagat          1654
Leu
505


gggaaaacca ttggggagga ctaggaccca tatgggaatt attacctctc agggccgaga      1714
ggacagaatg gatataatct gaatcctgtt aaattttctc taaactgttt cttagctgca      1774
ctgtttatgg aaataccagg accagtttat gtttgtggtt ttgggaaaaa ttatttgtgt      1834
tgggggaaat gttgtggggg tggggttgag ttggggggtat tttctaattt ttttttgtaca      1894
tttggaacag tgacaataaa tgagacccct ttaaactgtc aaaaaaaaaa aaaaaaaaaa      1954
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      2014
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      2074
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<210> 6
<211> 505
<212> PRT
<213> Homo sapiens

<400> 6

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            20                  25                  30
Asp Asn Phe Glu Ser Arg Ile Ser Asp Thr Gly Ser Ala Gly Leu Met
        35                  40                  45
Leu Val Glu Phe Phe Ala Pro Trp Cys Gly His Cys Lys Arg Leu Ala
    50                  55                  60
Pro Glu Tyr Glu Ala Ala Ala Thr Arg Leu Lys Gly Ile Val Pro Leu
65                  70                  75                  80
Ala Lys Val Asp Cys Thr Ala Asn Thr Asn Thr Cys Asn Lys Tyr Gly
                85                  90                  95
Val Ser Gly Tyr Pro Thr Leu Lys Ile Phe Arg Asp Gly Glu Glu Ala
            100                 105                 110
Gly Ala Tyr Asp Gly Pro Arg Thr Ala Asp Gly Ile Val Ser His Leu
        115                 120                 125
Lys Lys Gln Ala Gly Pro Ala Ser Val Pro Leu Arg Thr Glu Glu Glu
    130                 135                 140
Phe Lys Lys Phe Ile Ser Asp Lys Asp Ala Ser Ile Val Gly Phe Phe
145                 150                 155                 160
Asp Asp Ser Phe Ser Glu Ala His Ser Glu Phe Leu Lys Ala Ala Ser
                165                 170                 175
Asn Leu Arg Asp Asn Tyr Arg Phe Ala His Thr Asn Val Glu Ser Leu
            180                 185                 190
Val Asn Glu Tyr Asp Asp Asn Gly Glu Gly Ile Ile Leu Phe Arg Pro
            195                 200                 205
Ser His Leu Thr Asn Lys Phe Glu Asp Lys Thr Val Ala Tyr Thr Glu
    210                 215                 220
Gln Lys Met Thr Ser Gly Lys Ile Lys Lys Phe Ile Gln Glu Asn Ile
225                 230                 235                 240
Phe Gly Ile Cys Pro His Met Thr Glu Asp Asn Lys Asp Leu Ile Gln
                245                 250                 255
Gly Lys Asp Leu Leu Ile Ala Tyr Tyr Asp Val Asp Tyr Glu Lys Asn
            260                 265                 270
Ala Lys Gly Ser Asn Tyr Trp Arg Asn Arg Val Met Met Val Ala Lys
        275                 280                 285
Lys Phe Leu Asp Ala Gly His Lys Leu Asn Phe Ala Val Ala Ser Arg
    290                 295                 300
Lys Thr Phe Ser His Glu Leu Ser Asp Phe Gly Leu Glu Ser Thr Ala
305                 310                 315                 320
Gly Glu Ile Pro Val Val Ala Ile Arg Thr Ala Lys Gly Glu Lys Phe
                325                 330                 335
Val Met Gln Glu Glu Phe Ser Arg Asp Gly Lys Ala Leu Glu Arg Phe
            340                 345                 350
Leu Gln Asp Tyr Phe Asp Gly Asn Leu Lys Arg Tyr Leu Lys Ser Glu
        355                 360                 365
Pro Ile Pro Glu Ser Asn Asp Gly Pro Val Lys Val Val Val Ala Glu
    370                 375                 380
Asn Phe Asp Glu Ile Val Asn Asn Glu Asn Lys Asp Val Leu Ile Glu
385                 390                 395                 400
Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Asn Leu Glu Pro Lys Tyr
                405                 410                 415
Lys Glu Leu Gly Glu Lys Leu Ser Lys Asp Pro Asn Ile Val Ile Ala
            420                 425                 430
Lys Met Asp Ala Thr Ala Asn Asp Val Pro Ser Pro Tyr Glu Val Arg
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```
            435                  440                  445
Gly Phe Pro Thr Ile Tyr Phe Ser Pro Ala Asn Lys Lys Leu Asn Pro
    450                  455                  460
Lys Lys Tyr Glu Gly Gly Arg Glu Leu Ser Asp Phe Ile Ser Tyr Leu
465                  470                  475                  480
Gln Arg Glu Ala Thr Asn Pro Pro Val Ile Gln Glu Glu Lys Pro Lys
                485                  490                  495
Lys Lys Lys Lys Ala Gln Glu Asp Leu
            500                  505
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<210> 7
<211> 1899
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (69)...(1319)

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ggcccgcc atg ctg cta tcc gtg ccg ctg ctg ctc ggc ctc ctc ggc ctg    110
         Met Leu Leu Ser Val Pro Leu Leu Leu Gly Leu Leu Gly Leu
         1               5                  10

gcc gtc gcc gag ccc gcc gtc tac ttc aag gag cag ttt ctg gac gga    158
Ala Val Ala Glu Pro Ala Val Tyr Phe Lys Glu Gln Phe Leu Asp Gly
15              20              25              30

gac ggg tgg act tcc cgc tgg atc gaa tcc aaa cac aag tca gat ttt    206
Asp Gly Trp Thr Ser Arg Trp Ile Glu Ser Lys His Lys Ser Asp Phe
            35              40              45

ggc aaa ttc gtt ctc agt tcc ggc aag ttc tac ggt gac gag gag aaa    254
Gly Lys Phe Val Leu Ser Ser Gly Lys Phe Tyr Gly Asp Glu Glu Lys
            50              55              60

gat aaa ggt ttg cag aca agc cag gat gca cgc ttt tat gct ctg tcg    302
Asp Lys Gly Leu Gln Thr Ser Gln Asp Ala Arg Phe Tyr Ala Leu Ser
            65              70              75

gcc agt ttc gag cct ttc agc aac aaa ggc cag acg ctg gtg gtg cag    350
Ala Ser Phe Glu Pro Phe Ser Asn Lys Gly Gln Thr Leu Val Val Gln
    80              85              90

ttc acg gtg aaa cat gag cag aac atc gac tgt ggg ggc ggc tat gtg    398
Phe Thr Val Lys His Glu Gln Asn Ile Asp Cys Gly Gly Gly Tyr Val
95              100             105             110

aag ctg ttt cct aat agt ttg gac cag aca gac atg cac gga gac tca    446
Lys Leu Phe Pro Asn Ser Leu Asp Gln Thr Asp Met His Gly Asp Ser
            115             120             125

gaa tac aac atc atg ttt ggt ccc gac atc tgt ggc cct ggc acc aag    494
Glu Tyr Asn Ile Met Phe Gly Pro Asp Ile Cys Gly Pro Gly Thr Lys
            130             135             140

aag gtt cat gtc atc ttc aac tac aag ggc aag aac gtg ctg atc aac    542
Lys Val His Val Ile Phe Asn Tyr Lys Gly Lys Asn Val Leu Ile Asn
            145             150             155
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aag gac atc cgt tgc aag gat gat gag ttt aca cac ctg tac aca ctg    590
Lys Asp Ile Arg Cys Lys Asp Asp Glu Phe Thr His Leu Tyr Thr Leu
    160             165             170

att gtg cgg cca gac aac acc tat gag gtg aag att gac aac agc cag    638
Ile Val Arg Pro Asp Asn Thr Tyr Glu Val Lys Ile Asp Asn Ser Gln
175             180             185             190

gtg gag tcc ggc tcc ttg gaa gac gat tgg gac ttc ctg cca ccc aag    686
Val Glu Ser Gly Ser Leu Glu Asp Asp Trp Asp Phe Leu Pro Pro Lys
            195             200             205

aag ata aag gat cct gat gct tca aaa ccg gaa gac tgg gat gag cgg    734
Lys Ile Lys Asp Pro Asp Ala Ser Lys Pro Glu Asp Trp Asp Glu Arg
            210             215             220

gcc aag atc gat gat ccc aca gac tcc aag cct gag gac tgg gac aag    782
Ala Lys Ile Asp Asp Pro Thr Asp Ser Lys Pro Glu Asp Trp Asp Lys
        225             230             235

ccc gag cat atc cct gac cct gat gct aag aag ccc gag gac tgg gat    830
Pro Glu His Ile Pro Asp Pro Asp Ala Lys Lys Pro Glu Asp Trp Asp
    240             245             250

gaa gag atg gac gga gag tgg gaa ccc cca gtg att cag aac cct gag    878
Glu Glu Met Asp Gly Glu Trp Glu Pro Pro Val Ile Gln Asn Pro Glu
255             260             265             270

tac aag ggt gag tgg aag ccc cgg cag atc gac aac cca gat tac aag    926
Tyr Lys Gly Glu Trp Lys Pro Arg Gln Ile Asp Asn Pro Asp Tyr Lys
            275             280             285

ggc act tgg atc cac cca gaa att gac aac ccc gag tat tct ccc gat    974
Gly Thr Trp Ile His Pro Glu Ile Asp Asn Pro Glu Tyr Ser Pro Asp
            290             295             300

ccc agt atc tat gcc tat gat aac ttt ggc gtg ctg ggc ctg gac ctc   1022
Pro Ser Ile Tyr Ala Tyr Asp Asn Phe Gly Val Leu Gly Leu Asp Leu
            305             310             315

tgg cag gtc aag tct ggc acc atc ttt gac aac ttc ctc atc acc aac   1070
Trp Gln Val Lys Ser Gly Thr Ile Phe Asp Asn Phe Leu Ile Thr Asn
    320             325             330

gat gag gca tac gct gag gag ttt ggc aac gag acg tgg ggc gta aca   1118
Asp Glu Ala Tyr Ala Glu Glu Phe Gly Asn Glu Thr Trp Gly Val Thr
335             340             345             350

aag gca gca gag aaa caa atg aag gac aaa cag gac gag gag cag agg   1166
Lys Ala Ala Glu Lys Gln Met Lys Asp Lys Gln Asp Glu Glu Gln Arg
            355             360             365

ctt aag gag gag gaa gaa gac aag aaa cgc aaa gag gag gag gag gca   1214
Leu Lys Glu Glu Glu Glu Asp Lys Lys Arg Lys Glu Glu Glu Glu Ala
            370             375             380

gag gac aag gag gat gat gag gac aaa gat gag gat gag gag gat gag   1262
Glu Asp Lys Glu Asp Asp Glu Asp Lys Asp Glu Asp Glu Glu Asp Glu
            385             390             395

gag gac aag gag gaa gat gag gag gaa gat gtc ccc ggc cag gcc aag   1310
```

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Glu Asp Lys Glu Glu Asp Glu Glu Glu Asp Val Pro Gly Gln Ala Lys
    400                 405                 410


gac gag ctg tagagaggcc tgcctccagg gctggactga ggcctgagcg          1359
Asp Glu Leu
415


ctcctgccgc agagcttgcc gcgccaaata atgtctctgt gagactcgag aactttcatt   1419
tttttccagg ctggttcgga tttggggtgg attttggttt tgttcccctc ctccactctc   1479
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ctttctcccc tgcccccagg actgggccac ttctgggtgg ggcagtgggt cccagattgg   1839
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<210> 8
<211> 417
<212> PRT
<213> Homo sapiens


<400> 8
Met Leu Leu Ser Val Pro Leu Leu Leu Gly Leu Leu Gly Leu Ala Val
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Ala Glu Pro Ala Val Tyr Phe Lys Glu Gln Phe Leu Asp Gly Asp Gly
            20                  25                  30
Trp Thr Ser Arg Trp Ile Glu Ser Lys His Lys Ser Asp Phe Gly Lys
            35                  40                  45
Phe Val Leu Ser Ser Gly Lys Phe Tyr Gly Asp Glu Glu Lys Asp Lys
50                  55                  60
Gly Leu Gln Thr Ser Gln Asp Ala Arg Phe Tyr Ala Leu Ser Ala Ser
65                  70                  75                  80
Phe Glu Pro Phe Ser Asn Lys Gly Gln Thr Leu Val Val Gln Phe Thr
                85                  90                  95
Val Lys His Glu Gln Asn Ile Asp Cys Gly Gly Gly Tyr Val Lys Leu
            100                 105                 110
Phe Pro Asn Ser Leu Asp Gln Thr Asp Met His Gly Asp Ser Glu Tyr
            115                 120                 125
Asn Ile Met Phe Gly Pro Asp Ile Cys Gly Pro Gly Thr Lys Lys Val
130                 135                 140
His Val Ile Phe Asn Tyr Lys Gly Lys Asn Val Leu Ile Asn Lys Asp
145                 150                 155                 160
Ile Arg Cys Lys Asp Asp Glu Phe Thr His Leu Tyr Thr Leu Ile Val
                165                 170                 175
Arg Pro Asp Asn Thr Tyr Glu Val Lys Ile Asp Asn Ser Gln Val Glu
            180                 185                 190
Ser Gly Ser Leu Glu Asp Asp Trp Asp Phe Leu Pro Pro Lys Lys Ile
            195                 200                 205
Lys Asp Pro Asp Ala Ser Lys Pro Glu Asp Trp Asp Glu Arg Ala Lys
210                 215                 220
Ile Asp Asp Pro Thr Asp Ser Lys Pro Glu Asp Trp Asp Lys Pro Glu
225                 230                 235                 240
His Ile Pro Asp Pro Asp Ala Lys Lys Pro Glu Asp Trp Asp Glu Glu
                245                 250                 255
Met Asp Gly Glu Trp Glu Pro Pro Val Ile Gln Asn Pro Glu Tyr Lys
            260                 265                 270
Gly Glu Trp Lys Pro Arg Gln Ile Asp Asn Pro Asp Tyr Lys Gly Thr
            275                 280                 285
Trp Ile His Pro Glu Ile Asp Asn Pro Glu Tyr Ser Pro Asp Pro Ser
290                 295                 300
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Ile Tyr Ala Tyr Asp Asn Phe Gly Val Leu Gly Leu Asp Leu Trp Gln
305             310             315             320
Val Lys Ser Gly Thr Ile Phe Asp Asn Phe Leu Ile Thr Asn Asp Glu
                325             330             335
Ala Tyr Ala Glu Glu Phe Gly Asn Glu Thr Trp Gly Val Thr Lys Ala
            340             345             350
Ala Glu Lys Gln Met Lys Asp Lys Gln Asp Glu Glu Gln Arg Leu Lys
        355             360             365
Glu Glu Glu Glu Asp Lys Lys Arg Lys Glu Glu Glu Glu Ala Glu Asp
    370             375             380
Lys Glu Asp Asp Glu Asp Lys Asp Glu Asp Glu Glu Asp Glu Glu Asp
385             390             395             400
Lys Glu Glu Asp Glu Glu Glu Asp Val Pro Gly Gln Ala Lys Asp Glu
            405             410             415
Leu
```

```
<210> 9
<211> 1288
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (64)...(1215)

<400> 9
agaggcgcag agagagctgg gagctaaggg gtggcggcga ccggaagcgc agtgcacacc        60
ccc atg gcc cgg gct ttg gtc cag ttc tgg gcc ata tgc atg ctg cga       108
    Met Ala Arg Ala Leu Val Gln Phe Trp Ala Ile Cys Met Leu Arg
    1               5               10              15

gtg gcg ctg gct acc gtc tat ttc caa gag gaa ttt cta gac gga gag       156
Val Ala Leu Ala Thr Val Tyr Phe Gln Glu Glu Phe Leu Asp Gly Glu
                20              25              30

cat tgg aga aac cga tgg ttg cag tcc acc aat gac tcc cga ttt ggg       204
His Trp Arg Asn Arg Trp Leu Gln Ser Thr Asn Asp Ser Arg Phe Gly
            35              40              45

cat ttt aga ctt tcg tcg ggc aag ttt tat ggt cat aaa gag aaa gat       252
His Phe Arg Leu Ser Ser Gly Lys Phe Tyr Gly His Lys Glu Lys Asp
        50              55              60

aaa ggt ctg caa acc act cag aat ggc cga ttc tat gcc atc tct gca       300
Lys Gly Leu Gln Thr Thr Gln Asn Gly Arg Phe Tyr Ala Ile Ser Ala
    65              70              75

cgc ttc aaa ccg ttc agc aat aaa ggg aaa act ctg gtt att cag tac       348
Arg Phe Lys Pro Phe Ser Asn Lys Gly Lys Thr Leu Val Ile Gln Tyr
80              85              90              95

aca gta aaa cat gag cag aag atg gac tgt gga ggg ggc tac att aag       396
Thr Val Lys His Glu Gln Lys Met Asp Cys Gly Gly Gly Tyr Ile Lys
                100             105             110

gtc ttt cct gca gac att gac cag aag aac ctg aat gga aaa tcg caa       444
Val Phe Pro Ala Asp Ile Asp Gln Lys Asn Leu Asn Gly Lys Ser Gln
            115             120             125

tac tat att atg ttt gga ccc gat att tgt gga ttt gat atc aag aaa       492
```

```
Tyr Tyr Ile Met Phe Gly Pro Asp Ile Cys Gly Phe Asp Ile Lys Lys
        130             135             140

gtt cat gtt att tta cat ttc aag aat aag tat cac gaa aac aag aaa        540
Val His Val Ile Leu His Phe Lys Asn Lys Tyr His Glu Asn Lys Lys
        145             150             155

ctg atc agg tgt aag gtt gat ggc ttc aca cac ctg tac act cta att        588
Leu Ile Arg Cys Lys Val Asp Gly Phe Thr His Leu Tyr Thr Leu Ile
160             165             170             175

tta aga cca gat ctt tct tat gat gtg aaa att gat ggt cag tca att        636
Leu Arg Pro Asp Leu Ser Tyr Asp Val Lys Ile Asp Gly Gln Ser Ile
                180             185             190

gaa tcc ggc agc ata gag tac gac tgg aac tta aca tca ctc aag aag        684
Glu Ser Gly Ser Ile Glu Tyr Asp Trp Asn Leu Thr Ser Leu Lys Lys
            195             200             205

gaa acg tcc ccg gca gaa tcg aag gat tgg gaa cag act aaa gac aac        732
Glu Thr Ser Pro Ala Glu Ser Lys Asp Trp Glu Gln Thr Lys Asp Asn
            210             215             220

aaa gcc cag gac tgg gag aag cat ttt ctg gac gcc agc acc agc aag        780
Lys Ala Gln Asp Trp Glu Lys His Phe Leu Asp Ala Ser Thr Ser Lys
        225             230             235

cag agc gac tgg aac ggt gac ctg gat ggg gac tgg cca gcg ccg atg        828
Gln Ser Asp Trp Asn Gly Asp Leu Asp Gly Asp Trp Pro Ala Pro Met
240             245             250             255

ctc cag aag ccc ccg tac cag gat ggc ctg aaa cca gaa ggt att cat        876
Leu Gln Lys Pro Pro Tyr Gln Asp Gly Leu Lys Pro Glu Gly Ile His
                260             265             270

aaa gac gtc tgg ctc cac cgt aag atg aag aat acc gac tat ttg acg        924
Lys Asp Val Trp Leu His Arg Lys Met Lys Asn Thr Asp Tyr Leu Thr
                275             280             285

cag tat gac ctc tca gaa ttt gag aac att ggt gcc att ggc ctg gag        972
Gln Tyr Asp Leu Ser Glu Phe Glu Asn Ile Gly Ala Ile Gly Leu Glu
        290             295             300

ctt tgg cag gtg aga tct gga acc att ttt gat aac ttt ctg atc aca       1020
Leu Trp Gln Val Arg Ser Gly Thr Ile Phe Asp Asn Phe Leu Ile Thr
        305             310             315

gat gat gaa gag tat gca gat aat ttt ggc aag gcc acc tgg ggc gaa       1068
Asp Asp Glu Glu Tyr Ala Asp Asn Phe Gly Lys Ala Thr Trp Gly Glu
320             325             330             335

acc aag ggt cca gaa agg gag atg gat gcc ata cag gcc aag gag gaa       1116
Thr Lys Gly Pro Glu Arg Glu Met Asp Ala Ile Gln Ala Lys Glu Glu
                340             345             350

atg aag aag gcc cgc gag gaa gag gag gaa gag ctg ctg tcg gga aaa       1164
Met Lys Lys Ala Arg Glu Glu Glu Glu Glu Glu Leu Leu Ser Gly Lys
                355             360             365

att aac agg cac gaa cat tac ttc aat caa ttt cac aga agg aat gaa       1212
Ile Asn Arg His Glu His Tyr Phe Asn Gln Phe His Arg Arg Asn Glu
```

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                  370              375              380

ctt tagtgatccc cattggatat aaggatgact ggtaaaatct cattgctact          1265
Leu


ttaatctaaa aaaaaaaaaa aaa                                           1288
```

<210> 10
<211> 384
<212> PRT
<213> Homo sapiens

<400> 10

```
Met Ala Arg Ala Leu Val Gln Phe Trp Ala Ile Cys Met Leu Arg Val
1               5               10               15
Ala Leu Ala Thr Val Tyr Phe Gln Glu Glu Phe Leu Asp Gly Glu His
            20               25               30
Trp Arg Asn Arg Trp Leu Gln Ser Thr Asn Asp Ser Arg Phe Gly His
        35               40               45
Phe Arg Leu Ser Ser Gly Lys Phe Tyr Gly His Lys Glu Lys Asp Lys
    50               55               60
Gly Leu Gln Thr Thr Gln Asn Gly Arg Phe Tyr Ala Ile Ser Ala Arg
65               70               75               80
Phe Lys Pro Phe Ser Asn Lys Gly Lys Thr Leu Val Ile Gln Tyr Thr
                85               90               95
Val Lys His Glu Gln Lys Met Asp Cys Gly Gly Gly Tyr Ile Lys Val
            100              105              110
Phe Pro Ala Asp Ile Asp Gln Lys Asn Leu Asn Gly Lys Ser Gln Tyr
        115              120              125
Tyr Ile Met Phe Gly Pro Asp Ile Cys Gly Phe Asp Ile Lys Lys Val
    130              135              140
His Val Ile Leu His Phe Lys Asn Lys Tyr His Glu Asn Lys Lys Leu
145              150              155              160
Ile Arg Cys Lys Val Asp Gly Phe Thr His Leu Tyr Thr Leu Ile Leu
                165              170              175
Arg Pro Asp Leu Ser Tyr Asp Val Lys Ile Asp Gly Gln Ser Ile Glu
            180              185              190
Ser Gly Ser Ile Glu Tyr Asp Trp Asn Leu Thr Ser Leu Lys Lys Glu
        195              200              205
Thr Ser Pro Ala Glu Ser Lys Asp Trp Glu Gln Thr Lys Asp Asn Lys
    210              215              220
Ala Gln Asp Trp Glu Lys His Phe Leu Asp Ala Ser Thr Ser Lys Gln
225              230              235              240
Ser Asp Trp Asn Gly Asp Leu Asp Gly Asp Trp Pro Ala Pro Met Leu
                245              250              255
Gln Lys Pro Pro Tyr Gln Asp Gly Leu Lys Pro Glu Gly Ile His Lys
            260              265              270
Asp Val Trp Leu His Arg Lys Met Lys Asn Thr Asp Tyr Leu Thr Gln
        275              280              285
Tyr Asp Leu Ser Glu Phe Glu Asn Ile Gly Ala Ile Gly Leu Glu Leu
    290              295              300
Trp Gln Val Arg Ser Gly Thr Ile Phe Asp Asn Phe Leu Ile Thr Asp
305              310              315              320
Asp Glu Glu Tyr Ala Asp Asn Phe Gly Lys Ala Thr Trp Gly Glu Thr
                325              330              335
Lys Gly Pro Glu Arg Glu Met Asp Ala Ile Gln Ala Lys Glu Glu Met
            340              345              350
Lys Lys Ala Arg Glu Glu Glu Glu Glu Leu Leu Ser Gly Lys Ile
        355              360              365
Asn Arg His Glu His Tyr Phe Asn Gln Phe His Arg Arg Asn Glu Leu
```

16/30

370   375   380

<210> 11
<211> 1659
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (28)...(1560)

<400> 11
agcagtacag gcagaagctg gcggctc atg gct tcg tgc cca tgg ggt cag gaa   54
                                Met Ala Ser Cys Pro Trp Gly Gln Glu
                                1   5

cag gga gcg agg agc ccc tcg gag gag cct cca gag gag gaa atc ccc   102
Gln Gly Ala Arg Ser Pro Ser Glu Glu Pro Pro Glu Glu Glu Ile Pro
10   15   20   25

aag gag gat ggg atc ttg gtg ctg agc cgc cac acc ctg ggc ctg gcc   150
Lys Glu Asp Gly Ile Leu Val Leu Ser Arg His Thr Leu Gly Leu Ala
             30   35   40

ctg cgg gag cac cct gcc ctg ctg gtg gaa ttc tat gcc ccg tgg tgt   198
Leu Arg Glu His Pro Ala Leu Leu Val Glu Phe Tyr Ala Pro Trp Cys
             45   50   55

ggg cac tgc cag gcc ctg gcc ccc gag tac agc aag gca gct gcc gtg   246
Gly His Cys Gln Ala Leu Ala Pro Glu Tyr Ser Lys Ala Ala Ala Val
             60   65   70

ctc gcg gcc gag tca atg gtg gtc acg ctg gcc aag gtg gat ggg ccc   294
Leu Ala Ala Glu Ser Met Val Val Thr Leu Ala Lys Val Asp Gly Pro
             75   80   85

gcg cag cgc gag ctg gct gag gag ttt ggt gtg acg gag tac cct acg   342
Ala Gln Arg Glu Leu Ala Glu Glu Phe Gly Val Thr Glu Tyr Pro Thr
90   95   100   105

ctc aag ttc ttc cgc aat ggg aac cgc acg cac ccc gag gag tac aca   390
Leu Lys Phe Phe Arg Asn Gly Asn Arg Thr His Pro Glu Glu Tyr Thr
             110   115   120

gga cca cgg gac gct gag ggc att gcc gag tgg ctg cga cgg cgg gtg   438
Gly Pro Arg Asp Ala Glu Gly Ile Ala Glu Trp Leu Arg Arg Arg Val
             125   130   135

ggg ccc agt gcc atg cgg ctg gag gat gag gcg gcc gcc cag gcg ctg   486
Gly Pro Ser Ala Met Arg Leu Glu Asp Glu Ala Ala Ala Gln Ala Leu
             140   145   150

atc ggt ggc cgg gac cta gtg gtc att ggc ttc ttc cag gac ctg cag   534
Ile Gly Gly Arg Asp Leu Val Val Ile Gly Phe Phe Gln Asp Leu Gln
             155   160   165

gac gag gac gtg gcc acc ttc ttg gcc ttg gcc cag gac gcc ctg gac   582
Asp Glu Asp Val Ala Thr Phe Leu Ala Leu Ala Gln Asp Ala Leu Asp
170   175   180   185

atg acc ttt ggc ctc aca gac cgg ccg cgg ctc ttt cag cag ttt ggc   630

17/30

```
Met Thr Phe Gly Leu Thr Asp Arg Pro Arg Leu Phe Gln Gln Phe Gly
             190             195                 200

ctc acc aag gac act gtg gtt ctc ttc aag aag ttt gat gag ggg cgg    678
Leu Thr Lys Asp Thr Val Val Leu Phe Lys Lys Phe Asp Glu Gly Arg
             205             210                 215

gca gac ttc ccc gtg gac gag gag ctt ggc ctg gac ctg ggg gat ctg    726
Ala Asp Phe Pro Val Asp Glu Glu Leu Gly Leu Asp Leu Gly Asp Leu
             220             225                 230

tcg cgc ttc ctg gtc aca cac agc atg cgc ctg gtc acg gag ttc aac    774
Ser Arg Phe Leu Val Thr His Ser Met Arg Leu Val Thr Glu Phe Asn
             235             240                 245

agc cag acg tct gcc aag atc ttc gcg gcc agg atc ctc aac cac ctg    822
Ser Gln Thr Ser Ala Lys Ile Phe Ala Ala Arg Ile Leu Asn His Leu
250             255                 260                 265

ctg ctg ttt gtc aac cag acg ctg gct gcg cac cgg gag ctc cta gcg    870
Leu Leu Phe Val Asn Gln Thr Leu Ala Ala His Arg Glu Leu Leu Ala
             270             275                 280

ggc ttt ggg gag gca gct ccc cgc ttc cgg ggg cag gtg ctg ttc gtg    918
Gly Phe Gly Glu Ala Ala Pro Arg Phe Arg Gly Gln Val Leu Phe Val
             285             290                 295

gtg gtg gac gtg gcg gcc gac aat gag cac gtg ctg cag tac ttt gga    966
Val Val Asp Val Ala Ala Asp Asn Glu His Val Leu Gln Tyr Phe Gly
             300             305                 310

ctc aag gct gag gca gcc ccc act ctg cgc ttg gtc aac ctt gaa acc    1014
Leu Lys Ala Glu Ala Ala Pro Thr Leu Arg Leu Val Asn Leu Glu Thr
             315             320                 325

act aag aag tat gcg cct gtg gat ggg ggc cct gtc acc gca gcg tcc    1062
Thr Lys Lys Tyr Ala Pro Val Asp Gly Gly Pro Val Thr Ala Ala Ser
330             335                 340                 345

atc act gct ttc tgc cat gca gtc ctc aac ggc caa gtc aag ccc tat    1110
Ile Thr Ala Phe Cys His Ala Val Leu Asn Gly Gln Val Lys Pro Tyr
             350             355                 360

ctc ctg agc cag gag ata ccc cct gat tgg gat cag cgg cca gtt aag    1158
Leu Leu Ser Gln Glu Ile Pro Pro Asp Trp Asp Gln Arg Pro Val Lys
             365             370                 375

acc ctc gtg ggc aag aat ttt gag cag gtg gct ttt gac gaa acc aag    1206
Thr Leu Val Gly Lys Asn Phe Glu Gln Val Ala Phe Asp Glu Thr Lys
             380             385                 390

aat gtg ttt gtc aag ttc tat gcc ccg tgg tgc acc cac tgc aag gag    1254
Asn Val Phe Val Lys Phe Tyr Ala Pro Trp Cys Thr His Cys Lys Glu
             395             400                 405

atg gcc cct gcc tgg gag gca ttg gct gag aag tac caa gac cac gag    1302
Met Ala Pro Ala Trp Glu Ala Leu Ala Glu Lys Tyr Gln Asp His Glu
410             415                 420                 425

gac atc atc att gct gag ctg gat gcc acg gcc aac gag ctg gat gcc    1350
Asp Ile Ile Ile Ala Glu Leu Asp Ala Thr Ala Asn Glu Leu Asp Ala
```

```
                        430                 435                 440
ttc gct gtg cac ggc ttc cct act ctc aag tac ttc cca gca ggg cca      1398
Phe Ala Val His Gly Phe Pro Thr Leu Lys Tyr Phe Pro Ala Gly Pro
                445                 450                 455

ggt cgg aag gtg att gaa tac aaa agc acc agg gac ctg gag act ttc      1446
Gly Arg Lys Val Ile Glu Tyr Lys Ser Thr Arg Asp Leu Glu Thr Phe
                460                 465                 470

tcc aag ttc ctg gac aac ggg ggc gtg ctg ccc acg gag gag tcc ccg      1494
Ser Lys Phe Leu Asp Asn Gly Gly Val Leu Pro Thr Glu Glu Ser Pro
                475                 480                 485

gag gag cca gca gcc ccg ttc ccg gag cca ccg gcc aac tcc act atg      1542
Glu Glu Pro Ala Ala Pro Phe Pro Glu Pro Pro Ala Asn Ser Thr Met
490                 495                 500                 505

ggg tcc aag gag gaa ctg tagctgcccc cgtgtcaccc ccgccatcac      1590
Gly Ser Lys Glu Glu Leu
                510

tgctggacag gagccacccc cttgggtacc agagggagct gtgcattgtg aataaagagt      1650
gagcttggt                                                              1659
```

```
<210> 12
<211> 511
<212> PRT
<213> Homo sapiens

<400> 12
Met Ala Ser Cys Pro Trp Gly Gln Glu Gln Gly Ala Arg Ser Pro Ser
1               5                   10                  15
Glu Glu Pro Pro Glu Glu Glu Ile Pro Lys Glu Asp Gly Ile Leu Val
                20                  25                  30
Leu Ser Arg His Thr Leu Gly Leu Ala Leu Arg Glu His Pro Ala Leu
            35                  40                  45
Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Gln Ala Leu Ala
        50                  55                  60
Pro Glu Tyr Ser Lys Ala Ala Ala Val Leu Ala Ala Glu Ser Met Val
65                  70                  75                  80
Val Thr Leu Ala Lys Val Asp Gly Pro Ala Gln Arg Glu Leu Ala Glu
                85                  90                  95
Glu Phe Gly Val Thr Glu Tyr Pro Thr Leu Lys Phe Phe Arg Asn Gly
                100                 105                 110
Asn Arg Thr His Pro Glu Glu Tyr Thr Gly Pro Arg Asp Ala Glu Gly
            115                 120                 125
Ile Ala Glu Trp Leu Arg Arg Arg Val Gly Pro Ser Ala Met Arg Leu
        130                 135                 140
Glu Asp Glu Ala Ala Ala Gln Ala Leu Ile Gly Gly Arg Asp Leu Val
145                 150                 155                 160
Val Ile Gly Phe Phe Gln Asp Leu Gln Asp Glu Asp Val Ala Thr Phe
                165                 170                 175
Leu Ala Leu Ala Gln Asp Ala Leu Asp Met Thr Phe Gly Leu Thr Asp
                180                 185                 190
Arg Pro Arg Leu Phe Gln Gln Phe Gly Leu Thr Lys Asp Thr Val Val
            195                 200                 205
Leu Phe Lys Lys Phe Asp Glu Gly Arg Ala Asp Phe Pro Val Asp Glu
        210                 215                 220
Glu Leu Gly Leu Asp Leu Gly Asp Leu Ser Arg Phe Leu Val Thr His
225                 230                 235                 240
```

```
Ser Met Arg Leu Val Thr Glu Phe Asn Ser Gln Thr Ser Ala Lys Ile
            245             250             255
Phe Ala Ala Arg Ile Leu Asn His Leu Leu Leu Phe Val Asn Gln Thr
            260             265             270
Leu Ala Ala His Arg Glu Leu Leu Ala Gly Phe Gly Glu Ala Ala Pro
        275             280             285
Arg Phe Arg Gly Gln Val Leu Phe Val Val Val Asp Val Ala Ala Asp
    290             295             300
Asn Glu His Val Leu Gln Tyr Phe Gly Leu Lys Ala Glu Ala Ala Pro
305             310             315             320
Thr Leu Arg Leu Val Asn Leu Glu Thr Thr Lys Lys Tyr Ala Pro Val
            325             330             335
Asp Gly Gly Pro Val Thr Ala Ala Ser Ile Thr Ala Phe Cys His Ala
            340             345             350
Val Leu Asn Gly Gln Val Lys Pro Tyr Leu Leu Ser Gln Glu Ile Pro
            355             360             365
Pro Asp Trp Asp Gln Arg Pro Val Lys Thr Leu Val Gly Lys Asn Phe
    370             375             380
Glu Gln Val Ala Phe Asp Glu Thr Lys Asn Val Phe Val Lys Phe Tyr
385             390             395             400
Ala Pro Trp Cys Thr His Cys Lys Glu Met Ala Pro Ala Trp Glu Ala
            405             410             415
Leu Ala Glu Lys Tyr Gln Asp His Glu Asp Ile Ile Ile Ala Glu Leu
            420             425             430
Asp Ala Thr Ala Asn Glu Leu Asp Ala Phe Ala Val His Gly Phe Pro
        435             440             445
Thr Leu Lys Tyr Phe Pro Ala Gly Pro Gly Arg Lys Val Ile Glu Tyr
    450             455             460
Lys Ser Thr Arg Asp Leu Glu Thr Phe Ser Lys Phe Leu Asp Asn Gly
465             470             475             480
Gly Val Leu Pro Thr Glu Glu Ser Pro Glu Glu Pro Ala Ala Pro Phe
            485             490             495
Pro Glu Pro Pro Ala Asn Ser Thr Met Gly Ser Lys Glu Glu Leu
            500             505             510
```

```
<210> 13
<211> 2865
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (46)...(1980)

<400> 13
ccagcggccg ccgacgctag gaggccgcgc tccgcccccg ctacc atg agg ccc cgg    57
                                                    Met Arg Pro Arg
                                                    1


aaa gcc ttc ctg ctc ctg ctg ctc ttg ggg ctg gtg cag ctg ctg gcc    105
Lys Ala Phe Leu Leu Leu Leu Leu Leu Gly Leu Val Gln Leu Leu Ala
5                   10              15              20


gtg gcg ggt gcc gag ggc ccg gac gag gat tct tct aac aga gaa aat    153
Val Ala Gly Ala Glu Gly Pro Asp Glu Asp Ser Ser Asn Arg Glu Asn
                25              30              35


gcc att gag gat gaa gag gag gag gag gag gaa gat gat gat gag gaa    201
Ala Ile Glu Asp Glu Glu Glu Glu Glu Glu Glu Asp Asp Asp Glu Glu
            40              45              50
```

```
gaa gac gac ttg gaa gtt aag gaa gaa aat gga gtc ttg gtc cta aat          249
Glu Asp Asp Leu Glu Val Lys Glu Glu Asn Gly Val Leu Val Leu Asn
            55                      60                  65

gat gca aac ttt gat aat ttt gtg gct gac aaa gac aca gtg ctg ctg          297
Asp Ala Asn Phe Asp Asn Phe Val Ala Asp Lys Asp Thr Val Leu Leu
        70                  75                  80

gag ttt tat gct cca tgg tgt gga cat tgc aag cag ttt gct ccg gaa          345
Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Gln Phe Ala Pro Glu
85                  90                  95                  100

tat gaa aaa att gcc aac ata tta aag gat aaa gat cct ccc att cct          393
Tyr Glu Lys Ile Ala Asn Ile Leu Lys Asp Lys Asp Pro Pro Ile Pro
            105                 110                 115

gtt gcc aag atc gat gca acc tca gcg tct gtg ctg gcc agc agg ttt          441
Val Ala Lys Ile Asp Ala Thr Ser Ala Ser Val Leu Ala Ser Arg Phe
            120                 125                 130

gat gtg agt ggc tac ccc acc atc aag atc ctt aag aag ggg cag gct          489
Asp Val Ser Gly Tyr Pro Thr Ile Lys Ile Leu Lys Lys Gly Gln Ala
            135                 140                 145

gta gac tac gag ggc tcc aga acc cag gaa gaa att gtt gcc aag gtc          537
Val Asp Tyr Glu Gly Ser Arg Thr Gln Glu Glu Ile Val Ala Lys Val
150                 155                 160

aga gaa gtc tcc cag ccc gac tgg acg cct cca cca gaa gtc acg ctt          585
Arg Glu Val Ser Gln Pro Asp Trp Thr Pro Pro Pro Glu Val Thr Leu
165                 170                 175                 180

gtg ttg acc aaa gag aac ttt gat gaa gtt gtg aat gat gca gat atc          633
Val Leu Thr Lys Glu Asn Phe Asp Glu Val Val Asn Asp Ala Asp Ile
            185                 190                 195

att ctg gtg gag ttt tat gcc cca tgg tgt gga cac tgc aag aaa ctt          681
Ile Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Lys Leu
            200                 205                 210

gcc ccc gag tat gag aag gcc gcc aag gag ctc agc aag cgt tct cct          729
Ala Pro Glu Tyr Glu Lys Ala Ala Lys Glu Leu Ser Lys Arg Ser Pro
            215                 220                 225

cca att ccc ctg gca aag gtc gac gcc acc gca gaa aca gac ctg gcc          777
Pro Ile Pro Leu Ala Lys Val Asp Ala Thr Ala Glu Thr Asp Leu Ala
            230                 235                 240

aag agg ttt gat gtc tct ggc tat ccc acc ctg aaa att ttc cgc aaa          825
Lys Arg Phe Asp Val Ser Gly Tyr Pro Thr Leu Lys Ile Phe Arg Lys
245                 250                 255                 260

gga agg cct tat gac tac aac ggc cca cga gaa aaa tat gga atc gtt          873
Gly Arg Pro Tyr Asp Tyr Asn Gly Pro Arg Glu Lys Tyr Gly Ile Val
            265                 270                 275

gat tac atg atc gag cag tcc ggg cct ccc tcc aag gag att ctg acc          921
Asp Tyr Met Ile Glu Gln Ser Gly Pro Pro Ser Lys Glu Ile Leu Thr
            280                 285                 290

ctg aag cag gtc cag gag ttc ctg aag gat gga gac gat gtc atc atc          969
```

Leu Lys Gln Val Gln Glu Phe Leu Lys Asp Gly Asp Asp Val Ile Ile
        295   300   305

atc ggg gtc ttt aag ggg gag agt gac cca gcc tac cag caa tac cag   1017
Ile Gly Val Phe Lys Gly Glu Ser Asp Pro Ala Tyr Gln Gln Tyr Gln
        310   315   320

gat gcc gct aac aac ctg aga gaa gat tac aaa ttt cac cac act ttc   1065
Asp Ala Ala Asn Asn Leu Arg Glu Asp Tyr Lys Phe His His Thr Phe
325   330   335   340

agc aca gaa ata gca aag ttc ttg aaa gtc tcc cag ggg cag ttg gtt   1113
Ser Thr Glu Ile Ala Lys Phe Leu Lys Val Ser Gln Gly Gln Leu Val
                    345   350   355

gta atg cag cct gag aaa ttc cag tcc aag tat gag ccc cgg agc cac   1161
Val Met Gln Pro Glu Lys Phe Gln Ser Lys Tyr Glu Pro Arg Ser His
                360   365   370

atg atg gac gtc cag ggc tcc acc cag gac tcg gcc atc aag gac ttc   1209
Met Met Asp Val Gln Gly Ser Thr Gln Asp Ser Ala Ile Lys Asp Phe
            375   380   385

gtg ctg aag tac gcc ctg ccc ctg gtt ggc cac cgc aag gtg tca aac   1257
Val Leu Lys Tyr Ala Leu Pro Leu Val Gly His Arg Lys Val Ser Asn
        390   395   400

gat gct aag cgc tac acc agg cgc ccc ctg gtg gtc gtc tac tac agt   1305
Asp Ala Lys Arg Tyr Thr Arg Arg Pro Leu Val Val Val Tyr Tyr Ser
405   410   415   420

gtg gac ttc agc ttt gat tac aga gct gca act cag ttt tgg cgg agc   1353
Val Asp Phe Ser Phe Asp Tyr Arg Ala Ala Thr Gln Phe Trp Arg Ser
                425   430   435

aaa gtc cta gag gtg gcc aag gac ttc cct gag tac acc ttt gcc att   1401
Lys Val Leu Glu Val Ala Lys Asp Phe Pro Glu Tyr Thr Phe Ala Ile
                440   445   450

gcg gac gaa gag gac tat gct ggg gag gtg aag gac ctg ggg ctc agc   1449
Ala Asp Glu Glu Asp Tyr Ala Gly Glu Val Lys Asp Leu Gly Leu Ser
                455   460   465

gag agt ggg gag gat gtc aat gcc gcc atc ctg gac gag agt ggg aag   1497
Glu Ser Gly Glu Asp Val Asn Ala Ala Ile Leu Asp Glu Ser Gly Lys
        470   475   480

aag ttc gcc atg gag cca gag gag ttt gac tct gac acc ctc cgc gag   1545
Lys Phe Ala Met Glu Pro Glu Glu Phe Asp Ser Asp Thr Leu Arg Glu
485   490   495   500

ttt gtc act gct ttc aaa aaa gga aaa ctg aag cca gtc atc aaa tcc   1593
Phe Val Thr Ala Phe Lys Lys Gly Lys Leu Lys Pro Val Ile Lys Ser
                505   510   515

cag cca gtg ccc aag aac aac aag gga ccc gtc aag gtc gtg gtg gga   1641
Gln Pro Val Pro Lys Asn Asn Lys Gly Pro Val Lys Val Val Val Gly
                520   525   530

aag acc ttt gac tcc att gtg atg gac ccc aag aag gac gtc ctc atc   1689
Lys Thr Phe Asp Ser Ile Val Met Asp Pro Lys Lys Asp Val Leu Ile

```
                535                      540                      545

gag ttc tac gcg cca tgg tgc ggg cac tgc aag cag cta gag ccc gtg    1737
Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Gln Leu Glu Pro Val
     550                      555                      560

tac aac agc ctg gcc aag aag tac aag ggc caa aag ggc ctg gtc atc    1785
Tyr Asn Ser Leu Ala Lys Lys Tyr Lys Gly Gln Lys Gly Leu Val Ile
565                      570                      575                      580

gcc aag atg gac gcc act gcc aac gac gtc ccc agc gac cgc tat aag    1833
Ala Lys Met Asp Ala Thr Ala Asn Asp Val Pro Ser Asp Arg Tyr Lys
                585                      590                      595

gtg gag ggc ttc ccc acc atc tac ttc gcc ccc agt ggg gac aaa aag    1881
Val Glu Gly Phe Pro Thr Ile Tyr Phe Ala Pro Ser Gly Asp Lys Lys
                600                      605                      610

aac cca gtt aaa ttt gag ggt gga gac aga gat ctg gag cat ttg agc    1929
Asn Pro Val Lys Phe Glu Gly Gly Asp Arg Asp Leu Glu His Leu Ser
                615                      620                      625

aag ttt ata gaa gaa cat gcc aca aaa ctg agc agg acc aag gaa gag    1977
Lys Phe Ile Glu Glu His Ala Thr Lys Leu Ser Arg Thr Lys Glu Glu
     630                      635                      640

ctt tgaaggcctg aggtctgcgg aaggtgggag gaggcagacg ccctgcgtgg         2030
Leu
645

cccatggtcg gggcgtccac cggaggccgg caacaaacga cagtatctcg gattcctttt    2090
tttttttttt taattttta tactttgttg tttcacttca tgctctgaat actgaataac     2150
catgaatgac tgaatagttt agtccagatt tttacagagg atacatctat ttttatcatt     2210
atttggggtt tgaaaaattt ttttttacac cttctaattt ctttatttct caaagcagat     2270
aattcttctg tgtgaaaatg ttttcttttt ttaatttaag gtttaaaatt ccttttccaa     2330
atcatgttga ttttgctctt taaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaga     2390
agggctggga ccaaccgggt gagatccaca agtctctgga tgtggctgaa ggcaaataca     2450
caattgaagt actttctgtt ttgaagtgct ttcccttttg aatctggttt gaaacatgca     2510
gcttctgtct ctagcccaag gaaagaccaa aacataggga aataaaagca tttatctttg     2570
tcttggaagt aattgttgaa gttgtgcagt tgatcagtgc acagttagct gcaatgttta     2630
tagaaattga ttgttaaacc aaatttacac tggcatgtgt ggtgtagttt ctaaaaggca     2690
cttcacattt gaaatttttc ttaccttaga aagtttctag tgatctaaat gtctagtttt     2750
gtattctttt gtgtgtgttc actgtttctc agtattacca cttgaataat tctctgtaca     2810
ggggggtttg tgctatacac tgggatgtct aattgcagca ataaagcctt tcttt         2865
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<210> 14
<211> 645
<212> PRT
<213> Homo sapiens

<400> 14
Met Arg Pro Arg Lys Ala Phe Leu Leu Leu Leu Leu Gly Leu Val
1               5                   10                  15
Gln Leu Leu Ala Val Ala Gly Ala Glu Gly Pro Asp Glu Asp Ser Ser
             20                  25                  30
Asn Arg Glu Asn Ala Ile Glu Asp Glu Glu Glu Glu Glu Glu Asp
         35                  40                  45
Asp Asp Glu Glu Glu Asp Asp Leu Glu Val Lys Glu Glu Asn Gly Val
     50                  55                  60
Leu Val Leu Asn Asp Ala Asn Phe Asp Asn Phe Val Ala Asp Lys Asp
65                  70                  75                  80
```

```
Thr Val Leu Leu Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Gln
              85                      90                  95
Phe Ala Pro Glu Tyr Glu Lys Ile Ala Asn Ile Leu Lys Asp Lys Asp
            100                 105                 110
Pro Pro Ile Pro Val Ala Lys Ile Asp Ala Thr Ser Ala Ser Val Leu
            115                 120                 125
Ala Ser Arg Phe Asp Val Ser Gly Tyr Pro Thr Ile Lys Ile Leu Lys
    130                 135                 140
Lys Gly Gln Ala Val Asp Tyr Glu Gly Ser Arg Thr Gln Glu Glu Ile
145                 150                 155                 160
Val Ala Lys Val Arg Glu Val Ser Gln Pro Asp Trp Thr Pro Pro Pro
                165                 170                 175
Glu Val Thr Leu Val Leu Thr Lys Glu Asn Phe Asp Glu Val Val Asn
            180                 185                 190
Asp Ala Asp Ile Ile Leu Val Glu Phe Tyr Ala Pro Trp Cys Gly His
            195                 200                 205
Cys Lys Lys Leu Ala Pro Glu Tyr Glu Lys Ala Ala Lys Glu Leu Ser
    210                 215                 220
Lys Arg Ser Pro Pro Ile Pro Leu Ala Lys Val Asp Ala Thr Ala Glu
225                 230                 235                 240
Thr Asp Leu Ala Lys Arg Phe Asp Val Ser Gly Tyr Pro Thr Leu Lys
              245                 250                 255
Ile Phe Arg Lys Gly Arg Pro Tyr Asp Tyr Asn Gly Pro Arg Glu Lys
              260                 265                 270
Tyr Gly Ile Val Asp Tyr Met Ile Glu Gln Ser Gly Pro Pro Ser Lys
        275                 280                 285
Glu Ile Leu Thr Leu Lys Gln Val Gln Glu Phe Leu Lys Asp Gly Asp
    290                 295                 300
Asp Val Ile Ile Ile Gly Val Phe Lys Gly Glu Ser Asp Pro Ala Tyr
305                 310                 315                 320
Gln Gln Tyr Gln Asp Ala Ala Asn Asn Leu Arg Glu Asp Tyr Lys Phe
                325                 330                 335
His His Thr Phe Ser Thr Glu Ile Ala Lys Phe Leu Lys Val Ser Gln
            340                 345                 350
Gly Gln Leu Val Val Met Gln Pro Glu Lys Phe Gln Ser Lys Tyr Glu
    355                 360                 365
Pro Arg Ser His Met Met Asp Val Gln Gly Ser Thr Gln Asp Ser Ala
    370                 375                 380
Ile Lys Asp Phe Val Leu Lys Tyr Ala Leu Pro Leu Val Gly His Arg
385                 390                 395                 400
Lys Val Ser Asn Asp Ala Lys Arg Tyr Thr Arg Arg Pro Leu Val Val
                405                 410                 415
Val Tyr Tyr Ser Val Asp Phe Ser Phe Asp Tyr Arg Ala Ala Thr Gln
            420                 425                 430
Phe Trp Arg Ser Lys Val Leu Glu Val Ala Lys Asp Phe Pro Glu Tyr
        435                 440                 445
Thr Phe Ala Ile Ala Asp Glu Glu Asp Tyr Ala Gly Glu Val Lys Asp
    450                 455                 460
Leu Gly Leu Ser Glu Ser Gly Glu Asp Val Asn Ala Ala Ile Leu Asp
465                 470                 475                 480
Glu Ser Gly Lys Lys Phe Ala Met Glu Pro Glu Glu Phe Asp Ser Asp
                485                 490                 495
Thr Leu Arg Glu Phe Val Thr Ala Phe Lys Lys Gly Lys Leu Lys Pro
            500                 505                 510
Val Ile Lys Ser Gln Pro Val Pro Lys Asn Asn Lys Gly Pro Val Lys
            515                 520                 525
Val Val Val Gly Lys Thr Phe Asp Ser Ile Val Met Asp Pro Lys Lys
    530                 535                 540
Asp Val Leu Ile Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Gln
545                 550                 555                 560
Leu Glu Pro Val Tyr Asn Ser Leu Ala Lys Lys Tyr Lys Gly Gln Lys
```

```
                    565                    570                    575
Gly Leu Val Ile Ala Lys Met Asp Ala Thr Ala Asn Asp Val Pro Ser
                580                    585                    590
Asp Arg Tyr Lys Val Glu Gly Phe Pro Thr Ile Tyr Phe Ala Pro Ser
            595                    600                    605
Gly Asp Lys Lys Asn Pro Val Lys Phe Glu Gly Gly Asp Arg Asp Leu
        610                    615                    620
Glu His Leu Ser Lys Phe Ile Glu Glu His Ala Thr Lys Leu Ser Arg
625                    630                    635                    640
Thr Lys Glu Glu Leu
                645
```

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<210> 15
<211> 2412
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(2409)

<400> 15
atg agg gcc ctg tgg gtg ctg ggc ctc tgc tgc gtc ctg ctg acc ttc      48
Met Arg Ala Leu Trp Val Leu Gly Leu Cys Cys Val Leu Leu Thr Phe
1               5                  10                  15

ggg tcg gtc aga gct gac gat gaa gtt gat gtg gat ggt aca gta gaa      96
Gly Ser Val Arg Ala Asp Asp Glu Val Asp Val Asp Gly Thr Val Glu
                20                  25                  30

gag gat ctg ggt aaa agt aga gaa gga tca agg acg gat gat gaa gta     144
Glu Asp Leu Gly Lys Ser Arg Glu Gly Ser Arg Thr Asp Asp Glu Val
            35                  40                  45

gta cag aga gag gaa gaa gct att cag ttg gat gga tta aat gca tca     192
Val Gln Arg Glu Glu Glu Ala Ile Gln Leu Asp Gly Leu Asn Ala Ser
        50                  55                  60

caa ata aga gaa ctt aga gag aag tcg gaa aag ttt gcc ttc caa gcc     240
Gln Ile Arg Glu Leu Arg Glu Lys Ser Glu Lys Phe Ala Phe Gln Ala
65                  70                  75                  80

gaa gtt aac aga atg atg aaa ctt atc atc aat tca ttg tat aaa aat     288
Glu Val Asn Arg Met Met Lys Leu Ile Ile Asn Ser Leu Tyr Lys Asn
                85                  90                  95

aaa gag att ttc ctg aga gaa ctg att tca aat gct tct gat gct tta     336
Lys Glu Ile Phe Leu Arg Glu Leu Ile Ser Asn Ala Ser Asp Ala Leu
            100                 105                 110

gat aag ata agg cta ata tca ctg act gat gaa aat gct ctt tct gga     384
Asp Lys Ile Arg Leu Ile Ser Leu Thr Asp Glu Asn Ala Leu Ser Gly
            115                 120                 125

aat gag gaa cta aca gtc aaa att aag tgt gat aag gag aag aac ctg     432
Asn Glu Glu Leu Thr Val Lys Ile Lys Cys Asp Lys Glu Lys Asn Leu
        130                 135                 140

ctg cat gtc aca gac acc ggt gta gga atg acc aga gaa gag ttg gtt     480
Leu His Val Thr Asp Thr Gly Val Gly Met Thr Arg Glu Glu Leu Val
145                 150                 155                 160
```

```
aaa aac ctt ggt acc ata gcc aaa tct ggg aca agc gag ttt tta aac    528
Lys Asn Leu Gly Thr Ile Ala Lys Ser Gly Thr Ser Glu Phe Leu Asn
                165             170             175

aaa atg act gaa gca cag gaa gat ggc cag tca act tct gaa ttg att    576
Lys Met Thr Glu Ala Gln Glu Asp Gly Gln Ser Thr Ser Glu Leu Ile
                180             185             190

ggc cag ttt ggt gtc ggt ttc tat tcc gcc ttc ctt gta gca gat aag    624
Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Phe Leu Val Ala Asp Lys
                195             200             205

gtt att gtc act tca aaa cac aac aac gat acc cag cac atc tgg gag    672
Val Ile Val Thr Ser Lys His Asn Asn Asp Thr Gln His Ile Trp Glu
        210             215             220

tct gac tcc aat gaa ttt tct gta att gct gac cca aga gga aac act    720
Ser Asp Ser Asn Glu Phe Ser Val Ile Ala Asp Pro Arg Gly Asn Thr
225             230             235             240

cta gga cgg gga acg aca att acc ctt gtc tta aaa gaa gaa gca tct    768
Leu Gly Arg Gly Thr Thr Ile Thr Leu Val Leu Lys Glu Glu Ala Ser
                245             250             255

gat tac ctt gaa ttg gat aca att aaa aat ctc gtc aaa aaa tat tca    816
Asp Tyr Leu Glu Leu Asp Thr Ile Lys Asn Leu Val Lys Lys Tyr Ser
                260             265             270

cag ttc ata aac ttt cct att tat gta tgg agc agc aag act gaa act    864
Gln Phe Ile Asn Phe Pro Ile Tyr Val Trp Ser Ser Lys Thr Glu Thr
                275             280             285

gtt gag gag ccc atg gag gaa gaa gaa gca gcc aaa gaa gag aaa gaa    912
Val Glu Glu Pro Met Glu Glu Glu Glu Ala Ala Lys Glu Glu Lys Glu
        290                 295             300

gaa tct gat gat gaa gct gca gta gag gaa gaa gaa gaa gaa aag aaa    960
Glu Ser Asp Asp Glu Ala Ala Val Glu Glu Glu Glu Glu Glu Lys Lys
305                 310             315             320

cca aag act aaa aaa gtt gaa aaa act gtc tgg gac tgg gaa ctt atg   1008
Pro Lys Thr Lys Lys Val Glu Lys Thr Val Trp Asp Trp Glu Leu Met
                325             330             335

aat gat atc aaa cca ata tgg cag aga cca tca aaa gaa gta gaa gaa   1056
Asn Asp Ile Lys Pro Ile Trp Gln Arg Pro Ser Lys Glu Val Glu Glu
                340             345             350

gat gaa tac aaa gct ttc tac aaa tca ttt tca aag gaa agt gat gac   1104
Asp Glu Tyr Lys Ala Phe Tyr Lys Ser Phe Ser Lys Glu Ser Asp Asp
                355             360             365

ccc atg gct tat att cac ttt act gct gaa ggg gaa gtt acc ttc aaa   1152
Pro Met Ala Tyr Ile His Phe Thr Ala Glu Gly Glu Val Thr Phe Lys
                370             375             380

tca att tta ttt gta ccc aca tct gct cca cgt ggt ctg ttt gac gaa   1200
Ser Ile Leu Phe Val Pro Thr Ser Ala Pro Arg Gly Leu Phe Asp Glu
385                 390             395             400
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```
tat gga tct aaa aag agc gat tac att aag ctc tat gtg cgc cgt gta    1248
Tyr Gly Ser Lys Lys Ser Asp Tyr Ile Lys Leu Tyr Val Arg Arg Val
            405             410             415

ttc atc aca gac gac ttc cat gat atg atg cct aaa tac ctc aat ttt    1296
Phe Ile Thr Asp Asp Phe His Asp Met Met Pro Lys Tyr Leu Asn Phe
            420             425             430

gtc aag ggt gtg gtg gac tca gat gat ctc ccc ttg aat gtt tcc cgc    1344
Val Lys Gly Val Val Asp Ser Asp Asp Leu Pro Leu Asn Val Ser Arg
            435             440             445

gag act ctt cag caa cat aaa ctg ctt aag gtg att agg aag aag ctt    1392
Glu Thr Leu Gln Gln His Lys Leu Leu Lys Val Ile Arg Lys Lys Leu
            450             455             460

gtt cgt aaa acg ctg gac atg atc aag aag att gct gat gat aaa tac    1440
Val Arg Lys Thr Leu Asp Met Ile Lys Lys Ile Ala Asp Asp Lys Tyr
465             470             475             480

aat gat act ttt tgg aaa gaa ttt ggt acc aac atc aag ctt ggt gtg    1488
Asn Asp Thr Phe Trp Lys Glu Phe Gly Thr Asn Ile Lys Leu Gly Val
                485             490             495

att gaa gac cac tcg aat cga aca cgt ctt gct aaa ctt ctt agg ttc    1536
Ile Glu Asp His Ser Asn Arg Thr Arg Leu Ala Lys Leu Leu Arg Phe
            500             505             510

cag tct tct cat cat cca act gac att act agc cta gac cag tat gtg    1584
Gln Ser Ser His His Pro Thr Asp Ile Thr Ser Leu Asp Gln Tyr Val
            515             520             525

gaa aga atg aag gaa aaa caa gac aaa atc tac ttc atg gct ggg tcc    1632
Glu Arg Met Lys Glu Lys Gln Asp Lys Ile Tyr Phe Met Ala Gly Ser
            530             535             540

agc aga aaa gag gct gaa tct tct cca ttt gtt gag cga ctt ctg aaa    1680
Ser Arg Lys Glu Ala Glu Ser Ser Pro Phe Val Glu Arg Leu Leu Lys
545             550             555             560

aag ggc tat gaa gtt att tac ctc aca gaa cct gtg gat gaa tac tgt    1728
Lys Gly Tyr Glu Val Ile Tyr Leu Thr Glu Pro Val Asp Glu Tyr Cys
                565             570             575

att cag gcc ctt ccc gaa ttt gat ggg aag agg ttc cag aat gtt gcc    1776
Ile Gln Ala Leu Pro Glu Phe Asp Gly Lys Arg Phe Gln Asn Val Ala
            580             585             590

aag gaa gga gtg aag ttc gat gaa agt gag aaa act aag gag agt cgt    1824
Lys Glu Gly Val Lys Phe Asp Glu Ser Glu Lys Thr Lys Glu Ser Arg
            595             600             605

gaa gca gtt gag aaa gaa ttt gag cct ctg ctg aat tgg atg aaa gat    1872
Glu Ala Val Glu Lys Glu Phe Glu Pro Leu Leu Asn Trp Met Lys Asp
            610             615             620

aaa gcc ctt aag gac aag att gaa aag gct gtg gtg tct cag cgc ctg    1920
Lys Ala Leu Lys Asp Lys Ile Glu Lys Ala Val Val Ser Gln Arg Leu
625             630             635             640

aca gaa tct ccg tgt gct ttg gtg gcc agc cag tac gga tgg tct ggc    1968
```

```
Thr Glu Ser Pro Cys Ala Leu Val Ala Ser Gln Tyr Gly Trp Ser Gly
                645                 650                 655

aac atg gag aga atc atg aaa gca caa gcg tac caa acg ggc aag gac    2016
Asn Met Glu Arg Ile Met Lys Ala Gln Ala Tyr Gln Thr Gly Lys Asp
                660                 665                 670

atc tct aca aat tac tat gcg agt cag aag aaa aca ttt gaa att aat    2064
Ile Ser Thr Asn Tyr Tyr Ala Ser Gln Lys Lys Thr Phe Glu Ile Asn
                675                 680                 685

ccc aga cac ccg ctg atc aga gac atg ctt cga cga att aag gaa gat    2112
Pro Arg His Pro Leu Ile Arg Asp Met Leu Arg Arg Ile Lys Glu Asp
                690                 695                 700

gaa gat gat aaa aca gtt ttg gat ctt gct gtg gtt ttg ttt gaa aca    2160
Glu Asp Asp Lys Thr Val Leu Asp Leu Ala Val Val Leu Phe Glu Thr
705                 710                 715                 720

gca acg ctt cgg tca ggg tat ctt tta cca gac act aaa gca tat gga    2208
Ala Thr Leu Arg Ser Gly Tyr Leu Leu Pro Asp Thr Lys Ala Tyr Gly
                725                 730                 735

gat aga ata gaa aga atg ctt cgc ctc agt ttg aac att gac cct gat    2256
Asp Arg Ile Glu Arg Met Leu Arg Leu Ser Leu Asn Ile Asp Pro Asp
                740                 745                 750

gca aag gtg gaa gaa gag cct gaa gaa gaa cct gaa gag aca gca gaa    2304
Ala Lys Val Glu Glu Glu Pro Glu Glu Glu Pro Glu Glu Thr Ala Glu
                755                 760                 765

gac aca aca gaa gac aca gag caa gac gaa gat gaa gaa atg gat gtg    2352
Asp Thr Thr Glu Asp Thr Glu Gln Asp Glu Asp Glu Glu Met Asp Val
                770                 775                 780

gga aca gat gaa gaa gaa gaa aca gca aag gaa tct aca gct gaa aaa    2400
Gly Thr Asp Glu Glu Glu Glu Thr Ala Lys Glu Ser Thr Ala Glu Lys
785                 790                 795                 800

gat gaa ttg taa                                                    2412
Asp Glu Leu


<210> 16
<211> 803
<212> PRT
<213> Homo sapiens

<400> 16
Met Arg Ala Leu Trp Val Leu Gly Leu Cys Cys Val Leu Leu Thr Phe
1               5                   10                  15
Gly Ser Val Arg Ala Asp Asp Glu Val Asp Val Asp Gly Thr Val Glu
                20                  25                  30
Glu Asp Leu Gly Lys Ser Arg Glu Gly Ser Arg Thr Asp Asp Glu Val
                35                  40                  45
Val Gln Arg Glu Glu Glu Ala Ile Gln Leu Asp Gly Leu Asn Ala Ser
        50                  55                  60
Gln Ile Arg Glu Leu Arg Glu Lys Ser Glu Lys Phe Ala Phe Gln Ala
65                  70                  75                  80
Glu Val Asn Arg Met Met Lys Leu Ile Ile Asn Ser Leu Tyr Lys Asn
```

```
                      85                    90                    95
Lys Glu Ile Phe Leu Arg Glu Leu Ile Ser Asn Ala Ser Asp Ala Leu
                100                  105                  110
Asp Lys Ile Arg Leu Ile Ser Leu Thr Asp Glu Asn Ala Leu Ser Gly
            115                  120                  125
Asn Glu Glu Leu Thr Val Lys Ile Lys Cys Asp Lys Glu Lys Asn Leu
        130                  135                  140
Leu His Val Thr Asp Thr Gly Val Gly Met Thr Arg Glu Glu Leu Val
145                  150                  155                  160
Lys Asn Leu Gly Thr Ile Ala Lys Ser Gly Thr Ser Glu Phe Leu Asn
                165                  170                  175
Lys Met Thr Glu Ala Gln Glu Asp Gly Gln Ser Thr Ser Glu Leu Ile
            180                  185                  190
Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala Phe Leu Val Ala Asp Lys
        195                  200                  205
Val Ile Val Thr Ser Lys His Asn Asn Asp Thr Gln His Ile Trp Glu
    210                  215                  220
Ser Asp Ser Asn Glu Phe Ser Val Ile Ala Asp Pro Arg Gly Asn Thr
225                  230                  235                  240
Leu Gly Arg Gly Thr Thr Ile Thr Leu Val Leu Lys Glu Glu Ala Ser
                245                  250                  255
Asp Tyr Leu Glu Leu Asp Thr Ile Lys Asn Leu Val Lys Lys Tyr Ser
            260                  265                  270
Gln Phe Ile Asn Phe Pro Ile Tyr Val Trp Ser Ser Lys Thr Glu Thr
            275                  280                  285
Val Glu Glu Pro Met Glu Glu Glu Glu Ala Ala Lys Glu Glu Lys Glu
        290                  295                  300
Glu Ser Asp Asp Glu Ala Ala Val Glu Glu Glu Glu Glu Glu Lys Lys
305                  310                  315                  320
Pro Lys Thr Lys Lys Val Glu Lys Thr Val Trp Asp Trp Glu Leu Met
                325                  330                  335
Asn Asp Ile Lys Pro Ile Trp Gln Arg Pro Ser Lys Glu Val Glu Glu
            340                  345                  350
Asp Glu Tyr Lys Ala Phe Tyr Lys Ser Phe Ser Lys Glu Ser Asp Asp
        355                  360                  365
Pro Met Ala Tyr Ile His Phe Thr Ala Glu Gly Glu Val Thr Phe Lys
    370                  375                  380
Ser Ile Leu Phe Val Pro Thr Ser Ala Pro Arg Gly Leu Phe Asp Glu
385                  390                  395                  400
Tyr Gly Ser Lys Lys Ser Asp Tyr Ile Lys Leu Tyr Val Arg Arg Val
                405                  410                  415
Phe Ile Thr Asp Asp Phe His Asp Met Met Pro Lys Tyr Leu Asn Phe
            420                  425                  430
Val Lys Gly Val Val Asp Ser Asp Asp Leu Pro Leu Asn Val Ser Arg
        435                  440                  445
Glu Thr Leu Gln Gln His Lys Leu Leu Lys Val Ile Arg Lys Lys Leu
    450                  455                  460
Val Arg Lys Thr Leu Asp Met Ile Lys Lys Ile Ala Asp Asp Lys Tyr
465                  470                  475                  480
Asn Asp Thr Phe Trp Lys Glu Phe Gly Thr Asn Ile Lys Leu Gly Val
                485                  490                  495
Ile Glu Asp His Ser Asn Arg Thr Arg Leu Ala Lys Leu Leu Arg Phe
            500                  505                  510
Gln Ser Ser His His Pro Thr Asp Ile Thr Ser Leu Asp Gln Tyr Val
            515                  520                  525
Glu Arg Met Lys Glu Lys Gln Asp Lys Ile Tyr Phe Met Ala Gly Ser
        530                  535                  540
Ser Arg Lys Glu Ala Glu Ser Ser Pro Phe Val Glu Arg Leu Leu Lys
545                  550                  555                  560
Lys Gly Tyr Glu Val Ile Tyr Leu Thr Glu Pro Val Asp Glu Tyr Cys
                565                  570                  575
```

```
Ile Gln Ala Leu Pro Glu Phe Asp Gly Lys Arg Phe Gln Asn Val Ala
             580                 585                 590
Lys Glu Gly Val Lys Phe Asp Glu Ser Glu Lys Thr Lys Glu Ser Arg
         595                 600                 605
Glu Ala Val Glu Lys Glu Phe Glu Pro Leu Leu Asn Trp Met Lys Asp
     610                 615                 620
Lys Ala Leu Lys Asp Lys Ile Glu Lys Ala Val Val Ser Gln Arg Leu
 625                 630                 635                 640
Thr Glu Ser Pro Cys Ala Leu Val Ala Ser Gln Tyr Gly Trp Ser Gly
                 645                 650                 655
Asn Met Glu Arg Ile Met Lys Ala Gln Ala Tyr Gln Thr Gly Lys Asp
             660                 665                 670
Ile Ser Thr Asn Tyr Tyr Ala Ser Gln Lys Lys Thr Phe Glu Ile Asn
         675                 680                 685
Pro Arg His Pro Leu Ile Arg Asp Met Leu Arg Arg Ile Lys Glu Asp
     690                 695                 700
Glu Asp Asp Lys Thr Val Leu Asp Leu Ala Val Val Leu Phe Glu Thr
 705                 710                 715                 720
Ala Thr Leu Arg Ser Gly Tyr Leu Leu Pro Asp Thr Lys Ala Tyr Gly
                 725                 730                 735
Asp Arg Ile Glu Arg Met Leu Arg Leu Ser Leu Asn Ile Asp Pro Asp
             740                 745                 750
Ala Lys Val Glu Glu Glu Pro Glu Glu Glu Pro Glu Glu Thr Ala Glu
         755                 760                 765
Asp Thr Thr Glu Asp Thr Glu Gln Asp Glu Asp Glu Glu Met Asp Val
     770                 775                 780
Gly Thr Asp Glu Glu Glu Glu Thr Ala Lys Glu Ser Thr Ala Glu Lys
 785                 790                 795                 800
Asp Glu Leu
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<210> 17
<211> 21
<212> DNA
<213> Homo sapiens

<400> 17
aacaactgca tgggtaacct t                                              21
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